THE AMERICAN JOURNAL OF PATHOLOGY

VOLUME VI SEPTEMBER, 1930 NUMBER 5

THE SIGNIFICANCE OF THE MUSCULAR "STROMA" OF ARGENTAFFIN TUMORS (CARCINOIDS) *

P. MASSON

(From the Department of Pathological Anatomy, University of Montreal, Montreal, Canada)

The physiological rôle of the argentaffin cells remains absolutely unknown. From the beginning of my researches I had the idea, since expressed by Danisch, of using extracts of carcinoids in physiological experiments. Circumstances hitherto have not favored the realization of this project. In the meantime, may not a purely morphological study of carcinoids point the way.

We have long known that most carcinoids possess a stroma more or less rich in smooth muscle fibers. In his remarkable chapter devoted to these tumors, Oberndorfer [1] hesitates over the interpretation of these fibers. For him, it is not improbable that they come from the muscularis mucosae. "They have no connection whatever with the circular and longitudinal muscle coats and, for the opinion that they form an integral part of the tumor, there is much to be said (vieles für sich hat)." Comparison of several carcinoids of different degrees of development and of invasion will lead us to a more exact and perhaps more suggestive interpretation.

OBSERVATIONS

Obs. 1 (93 c): Diagram 1. Voluminous appendix with thickened walls. The thickening affects the submucosa almost exclusively.

In its entire length the appendix presents the lesions characteristic of hypertrophy of the musculonervous complex of the submucosa (Masson [2]). Fibers of the muscle coat voluminous, like those of a

* Received for publication April 30, 1930.

gravid uterus. Submucosa fibrous, traversed by voluminous muscle bundles that unite the circular muscle and the muscularis mucosae. Nerves and ganglia of Meissner's plexus hypertrophied. Muscularis mucosae notably hypertrophied.

The thick mucosa poor in lymph nodules. Crypts broad, elongated, the cells hypertrophied, most of them containing mucus in

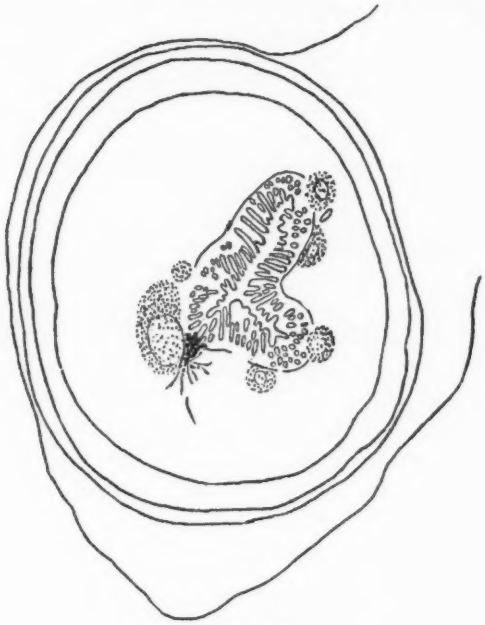


DIAGRAM 1

large calices. The stroma of the mucosa consists of the usual reticulum, and especially of a network of slender nerve fibers deprived of argentaffin cells. The lymph filling the meshes of this reticulum is poor in free cells.

Two areas, the tip and the middle of the appendix, present special features. At the tip of the appendix there is a voluminous argentaffin cell neuroma. This neuroma is so large that it has ruptured the muscularis mucosae and the muscle coats. It is capped by an attenuated submucosa which makes a hernia beneath the visceral peritoneum.

In the middle of the appendix, between the tips of three or four crypts and the slightly bulging muscularis mucosae, is a group of typical carcinoid columns. These columns are slender; they consist of polygonal and especially of palisaded cells. The cytoplasm of these cells contains fine acidophilic granules gathered at one pole. They do not reduce silver.* Some of them have two nuclei, probably an indication of amitotic division.

The carcinoid columns anastomose in a plexus. They are enclosed in a thin sheath of collagen. It can be seen easily that they are not situated in the lymphatic interstices of the mucosa but in the nerves themselves; they have penetrated the nerves and adopted their plexiform structure. Alongside of the area that contains the carcinoid cells, the nerves are slightly hypertrophied and pressed more closely together than in the rest of the appendix.

These columns occupy a very restricted area of the mucosa; some of them send prolongations into the nerves of the muscularis mucosae; they are found even as far as the superficial filaments of the submucous plexus. It is obvious that the carcinoid, intranervous in its entire extent, has arisen in the subglandular nerves and that the invasion of the nerves of the muscularis mucosae and of the submucosa is comparatively recent.

The contractile cells of this region are slightly hypertrophied but in this they do not differ noticeably from those of the other regions of the appendix. It is obvious that they were there before the appearance of the tumor and that they do not form an integral part of it in any sense.

Obs. 2 (241 h): Diagram 2. Appendix with uniform lumen. Cavity filled with pus.

The mucosa, rich in lymph nodules, five to seven per cross-section, presents no ulceration. Discrete infiltration with polynuclears in its superficial region. Deeper down, as in the submucosa and the muscle coats, there are no wandering cells. No peritoneal reaction. This appendix, then, was removed during a simple catarrhal inflammation which has not altered its structure in any way.

* I have stated elsewhere that the silver-reducing properties correspond to a functional state of the argentaffin cells; that the silver-reducing granules are not found in all of the cells of carcinoids; and that some of these tumors are completely free from them, although their nature cannot be doubted for all that. In the absence of these granules the carcinoid cells have enough other special characteristics to make their identification easy.

Throughout the appendix the subglandular plexus is moderately hypertrophied. Here and there are beginning neuromas. The nerve filaments contain round argentaffin cells, either single or in small groups, always few in number.

The submucosa is normal in thickness. The nerves of Meissner's plexus are voluminous and the number of muscle bundles is notably increased. There is nothing abnormal in Auerbach's plexus or in the

DIAGRAM 2

muscle coats. There is, then, both hyperplasia of the nerves of the mucosa containing argentaffin cells, and moderate hyperplasia of the musculonervous complexes of the submucosa.

Near the tip of the appendix, embedded in the submucosa and occupying its inner two-thirds, is a small sphere colored red by the trichrome stain. This minute tumor is continuous with the muscularis mucosae but it does not reach the circular muscle. Only a few slender bundles detach themselves from the circular muscle, as in all appendices, and fuse with the outer surface of the tumor. Examina-

tion with high magnification shows this tumor to be a myoma with cells elongated and running in all directions. Its interstices are occupied by collagen fibers, many capillaries, slender nerve filaments, and small clusters of argentaffin cells.

Between the myoma and the tips of the crypts of Lieberkühn is found a hypertrophied nerve plexus rich in argentaffin cells. This plexus is continuous with the rich and complex nerve network which ramifies among the fibers of the adjacent myoma. Without any possible doubt, the myoma is the result of hyperplasia of the muscularis mucosae.

The carcinoid columns included in this neuroma do not infiltrate the connective tissue, but they penetrate the nerve filaments and assume their plexiform arrangement. This penetration is not general. At certain points where the carcinoid columns are interrupted, they are seen to be continued by nerves which are intact but more slender, and it can be seen also that the carcinoid columns are separated from the surrounding tissue by a thin sheath of collagen which is continuous with that of the still uninvaded nerve.

Without doubt, this carcinoid arose in the nerves of the mucosa. Its proliferating cells invaded the nerves of the muscularis mucosae and the contractile fibers of this muscle have multiplied, giving rise to a myoma which, from the mere fact of its origin, occupies the interstices of the invaded nerve plexus.

Obs. 3 (123 m): Diagram 3. Appendicular lumen regular but narrow and cylindrical. Lymphatic tissue quite abundant, five to ten nodules in each cross-section.

The subglandular nerve plexus contains a number of isolated argentaffin cells. This plexus is hyperplastic; here and there it forms tiny neuromas which push the muscularis mucosae outward. The submucosa is adipose; it presents muscle fibers and nerves that are moderately but distinctly hyperplastic. In short, along its whole length this appendix shows typical hyperplasia of the subglandular plexus containing many argentaffin cells, and hyperplasia of the musculonervous complex of the submucosa.

Near the tip there are special lesions which merit closer attention. Around four-fifths of its circumference the appendix has the characteristics just described, except that it is poorer in lymph nodules, two to each cross-section. The remaining fifth presents the following peculiarities.

In the mucosa and capping the tips of three crypts of Lieberkühn, slender carcinoid columns form an almost continuous plexus and penetrate a tiny intramucous neuroma. Outside of the invaded areas, the nerves are intact and have not undergone any degeneration, either above or below the penetrated points or between them. The adjacent portion of the muscularis mucosae is much thickened and forms a small fasciculated myoma which encroaches on half the thickness of the submucosa.

Laterally, this myoma of the muscularis mucosae anastomoses with another myoma occupying the outer half of the submucosa and

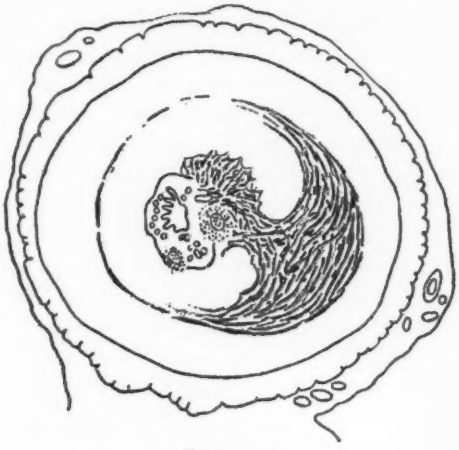


DIAGRAM 3

attached externally to the circular muscle. This latter myoma, conical in form, has a truncated summit by which it is continuous with the myoma of the muscularis mucosae; it seems to represent an intense hyperplasia of some of the muscle bundles of the submucosa. Its bundles are formed of large fibers rich in myofibrils. Its broad base corresponds to the middle zone of the circular muscle. In all of this external portion the contractile fibers are poorly differentiated, with few myofibrils, clear and oriented in all directions without appreciable bundle formation.

It is obvious that this myoma results from hyperplasia of preëxisting muscle fibers and not from the production of entirely new muscle fibers between the old bundles. If the latter condition obtained,

adult circular fibers would be found among the disoriented young fibers. Now, these latter alone are present to the exclusion of the others throughout the whole myoma which occupies the place of the circular muscle. Moreover, this area of young fibers is continuous externally by all possible transitions with the normal muscularis of adult circular fibers.

Summing up these various myomas, we find that each of them has for its origin a special muscle apparatus, that is, muscularis mucosae, muscle complex of the submucosa and circular muscle. We learn further that their muscle fibers are well differentiated and at rest in the myomas nearest the mucosa, young and in active proliferation in the myoma of the circular muscle. The latter is obviously more recent than the others.

The interstices of all these myomas are invaded by the carcinoid, the *neurocarcinoid*, originating in the mucosa, and here too the invasion is not into the connective tissue but purely nervous. The invasion is abundant enough to mark out the reticular structure of the affected plexus, discrete enough to permit recognition of the segments of intact nerves between the infiltrated portions, and so distinct that its limits can be determined precisely. The muscular hyperplasia is produced exclusively at the expense of the muscle coats, the nerves of which have been infiltrated; in the interstices of the mucous portion of the carcinoid there are no muscle fibers. It is most mature in the earliest invaded areas; for instance, in the muscularis mucosae it consists of adult muscle fibers, in the circular muscle of young fibers.

Obs. 4 (320 h): Diagram 4. Carcinoid of the tip of the appendix.

The carcinoid is separated from the end of the appendicular lumen by a voluminous, axial, argentaffin cell neuroma in which, beyond any doubt, it has arisen (neurocarcinoid). The tumor fills the appendicular axis and penetrates the circular muscle at a very restricted point, invading only the myenteric nerves and this in a very discrete manner. All of that region of the circular muscle around the infested nerves has undergone considerable hyperplasia, resulting in a lenticular-shaped myoma. This myoma, at its margin, is continuous with the circular muscle; it encroaches on and deforms the carcinoid.

Slender argentaffin columns gain the longitudinal muscle and cross it here and there around one-half of the circumference of the appen-

dix. Many, probably all of them, are intranervous. In all this region this coat is much thickened, whereas it is very thin on the opposite side where there are no argentaffin cells. It should be noted that at one point (upper part of the figure), the new fibers issuing from the external layer of the longitudinal coat pursue a very abnormal course; most of them are circular.

Having traversed the muscle coat, the tumor columns invade the serosa where they acquire a much larger caliber. In all of this outer zone they are not in the nerves but in a connective tissue normally deprived of muscle fibers. This tissue becomes denser between the

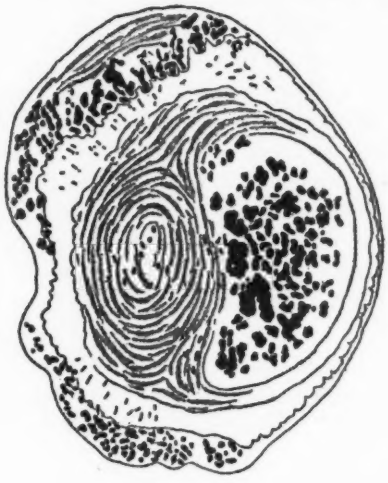


DIAGRAM 4

columns but there is not a single muscle fiber in the interstices. At this point, the stroma of the carcinoid contains no smooth muscle fibers.

Obs. 5 (Mag.): Diagram 5. Last centimeter of the appendix obliterated, occupied entirely by a carcinoid.

The tumor fills the axis and penetrates the muscle coat at a narrow point. From this point, the carcinoid columns spread through the interstices of the circular muscle around three-fourths of its circumference, leaving one-fourth free. Finally they gain the longitudinal muscle where they spread radially.

The stroma of the axial portion of the tumor is very fibrous but it

is easy to distinguish many smooth muscle fibers continuous with the vestiges of the muscularis mucosae.

In all of the muscle region invaded, nearly all, perhaps all, of the carcinoid cells are in the nerves and each of these coats presents notable thickening, maximum at the point of penetration of the circular muscle by the carcinoid, diminishing gradually at each side in the regions more recently invaded, to cease beyond the zone of invasion.

It should be noted further that a group of argentaffin columns have penetrated the nerves and perhaps the lymphatics which run

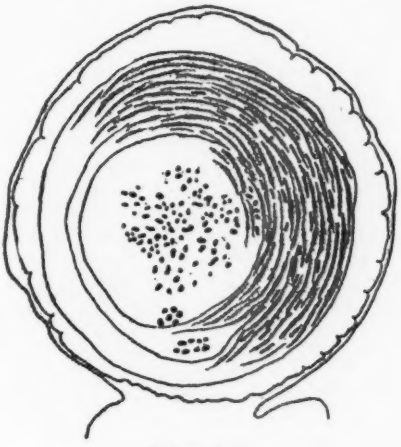


DIAGRAM 5

alongside of a large artery coming from the meso-appendix. These columns traverse the muscle coats without putting out prolongations into their nerves. Despite this propinquity, the muscle fibers do not undergo any change either of dimensions or number.

Obs. 6 (31 m): Diagram 6. Carcinoid at the tip of the appendix.

The nearly spherical tumor measures about 7 mm. in diameter. It is separated from the circular and longitudinal muscle by a thin submucosa. Excentrically placed, there is a small rounded group of voluminous carcinoid columns separated by purely connective tissue septa. This tiny object probably represents the axis of the obliterated appendix, the point of origin of the carcinoid, pushed to one side by the unequal growth of the rest of the tumor.

The tumor is an enormous myoma with very compact muscle fibers. There are three regions differing in the orientation of their fibers; in the middle the fibers are longitudinal; at the periphery they are circular; in the intermediate zone they are oblique. Judging from its situation within the submucosa, this myoma manifestly corresponds to an enormous hyperplasia of the muscularis mucosae. Its interstices are infiltrated by many carcinoid columns, most of them intranervous.

If we turn now to the muscular coats of the appendix, we find that for four-fifths of their circumference they are very thin and sepa-

DIAGRAM 6

rated from the central myocarcinoid by the submucosa. In the remaining fifth (left side of the figure) they are thick and the circular muscle is connected with the myoma by anastomotic fibers. In this region, and in this region only, Auerbach's plexus is infiltrated with carcinoid columns. Some of these columns pass beyond the plexus and the longitudinal muscle to invade the connective tissue of the visceral serosa. The stroma of this peritoneal portion of the tumor does not contain a single muscle cell.

Obs. 7 (102 f): Diagram 7. (In this diagram, the contour of the axial carcinoid is indicated by a festooned line.)

An appendix 6 cm. long, regularly cylindrical, except at the tip which is slightly swollen; at this point, the peritoneum is yellow and opaque, foretelling the presence of a carcinoid. In the first 3 cm. the appendix is permeable; in the last 3 cm. obliterated.

The permeable region need detain us little. In the mucosa, lymph nodules are few; between them, the subglandular plexus is slightly hyperplastic and contains a few argentaffin cells. There is no ab-

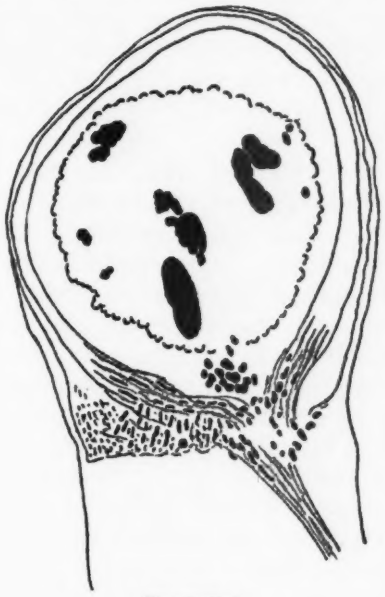


DIAGRAM 7

normality in the musculonervous complex of the submucosa nor in the muscle coats.

The distal four-fifths of the obliterated region is occupied by a carcinoid, the proximal fifth by a voluminous argentaffin cell axial neuroma, continuous with the plexus of the mucosa. The carcinoid invades the neuroma from below upward and we may suppose that it originated in the terminal region of this neuroma, which it replaces (neurocarcinoid).

The carcinoid fills the axis of the appendix but does not invade the submucosa, which is thinned and pushed before it. A discontinuous

muscle sheath, representing the muscularis mucosae, separates them. At one point only, the muscularis mucosae is traversed by carcinoid columns which penetrate the muscle coats along one-fifth of their circumference and gain the serosa. Attentive study of this zone of invasion shows that many of the carcinoid columns are not really in the interfascicular connective tissue but in the filaments of Auerbach's plexus.

In all of the invaded region the circular and the longitudinal muscle coats are thickened. The thickening doubtless depends in part on the infiltration of their interstices with foreign cells, but chiefly on the multiplication and hypertrophy of the contractile cells themselves. A little beyond the zone of invasion the thickening ceases rapidly.

We should note an important point. The carcinoid columns traverse the muscle coats, either through the nerves or through the interstitial tissue, to infiltrate the peritoneal nerve filaments and connective tissue. In these regions where there is no preëxistent muscle, the stroma of the tumor is pure connective tissue; but if, as in the section represented in the figure, the argentaffin cells following the nerves gain a muscle bundle belonging to the meso-appendix, this muscle bundle undergoes local hypertrophy identical with that of the appendicular muscle.

Discussion

(*a*) The small carcinoids which I have been able to observe in permeable appendices begin in tiny neuromas situated in the subglandular portion of the plexus of the mucosa. Several observations related in this paper, as well as others not reported here, lead me to believe that when carcinoids develop in previously obliterated appendices, they arise in axial neuromas resulting from the persistence or increase of the tiny neuromas already mentioned. All of the young carcinoids that I have seen are *neurocarcinoids*.

All of the proliferating argentaffin cells are of the glandular type. They penetrate the nerves electively without provoking either their increase (this was acquired previously) or their degeneration; therefore the cells are not neurogenic. If, then, the initial neuroma has been provoked by certain argentaffin cells, it is not by these glandular cells, for their multiplication has not this effect, but by others

which take no part in the constitution of the carcinoid, namely, by those cells which I have thought to be *ganglionic*.

After penetrating the intramucous neuroma the carcinoid columns gain the filaments that traverse the muscularis mucosae, then Meissner's plexus, then Auerbach's plexus. They infiltrate without destroying the nerve fibers or the ganglion cells of the myenteric plexus and without provoking their increase. In the meantime, some of them get out of the nerve and invade the connective tissue and even the lymphatics.

This intranervous progress is not peculiar to carcinoids; it is seen also in epitheliomas of the digestive tract; but, in this event, the invasion of the nerve is secondary, it follows interstitial invasion. In carcinoids, on the contrary, the penetration of the nerve is primary while the connective tissue invasion is secondary. The name *neuro-carcinoid* which I have proposed to give to carcinoids thus seems to me to be more justifiable than ever.

(*b*) Tracing the steps of this intranervous invasion we find that the corollary of the penetration of the various planes of the myenteric plexus is an overgrowth of the smooth muscle of the infested nervous region. To the invasion of the nerves of the muscularis mucosae there corresponds a myoma springing from it. If the carcinoid columns gain the nerves of the circular muscle and the submucous bundles depending on them, another myoma is added to the first. Similarly, when the carcinoid attains the external branches of Auerbach's plexus, the longitudinal muscle hypertrophies in its turn. If, in addition, the nerves of a muscle bundle of the meso-appendix are infiltrated, this bundle increases also. It should be noted that the overgrowth of the muscle is restricted to the area of nerve invasion; beyond this point it ceases quite abruptly.

Finally, we should note the chief point. When the carcinoid columns invade the nerves or the connective tissue of the visceral serosa or the fat of the meso-appendix, no muscle fibers are seen in their interstices. It follows that the muscle fibers observed in the "stroma" of carcinoids are not an integral part of these tumors. They result from proliferation of preëxisting muscle fibers, the nerves of which have been invaded by the carcinoid cells. The myogenic influence of the invasion of the nerves does not extend to a distance; it seems to exhaust itself on the spot. Moreover, it does not affect this or that muscle coat to the exclusion of the others, but it involves

every muscle region where the nerves are infested with argentaffin cells.

(*c*) What might be the cause of this muscular hypergenesis? Elsewhere [2] I have shown that the appendicular muscles, those of the muscularis mucosae, those of the submucosa and those of the muscle coats, may undergo a hyperplasia, the cause of which seems to be hyperplasia of the myenteric sympathetic. In the lesion under discussion, it is probable that here again the muscle hyperplasia is due to a nervous excitation but of quite another nature; it seems to be determined by the carcinoid invasion of the same plexuses.

One might think that the mere presence of foreign cells in the nerve filaments would excite them and provoke hyperplasia of the corresponding muscle territories. Were this true, invasion of the myenteric plexus by ordinary cancer cells should have a similar effect; but nothing like this is seen. May we not suppose, then, that the muscle hyperplasia is incited by a special intranervous secretion, *a neurocrinia* [3] of the carcinoid cells?

What is this substance secreted by the argentaffin cells? In these cells are found two kinds of inclusions, fats and granules. The fats are complex; their microchemical characters show that they consist of a mixture of neutral fats and cholesterin ethers in variable proportions. In argentaffin cell neuromas I have observed that these fats elaborated in the cells may be eliminated directly into the nerve filaments where they soon disappear, as if they had been utilized by the nerve tissue. The argentaffin granules are as chromaffin as are those of the paraganglia, hence the idea proposed by Ehrlich,[4] by myself,[5] and subsequently by Danisch,[6] that the carcinoids are phaeochromoblastomas or paragangliomas comparable to the tumors of the adrenal medulla.

Ehrlich and Danisch believed that the carcinoids were sympathetic phaeochromoblastomas analogous to those of the adrenal. The certain endodermic origin of the carcinoid cells, their argentaffinity together with their chromaffinity, have led me to an opinion which is at the same time similar and different. For me they are paragangliomas originating in a nervous system derived from the entoderm, a neurentoderm. Be that as it may, the chromaffinity of these cells permits us to suppose that, in addition to the fats, they secrete a substance more or less comparable to adrenalin but nevertheless different. We may suppose that this substance, poured into the

nerves, excites them and causes hypertrophy of the corresponding muscles.

This leads us back to a hypothesis formerly proposed by Ciaccio [7] of the rôle of the normal enterochromaffin cells. He believed that these cells secrete into the intestinal lumen a substance which, reabsorbed by the mucosa, played a part in the contraction of the intestinal muscle. What we have learned of the neurotropism of these cells enables us to modify this hypothesis somewhat. May we not suppose that their secretion, eliminated into the nerves of the mucosa (its limited diffusion is demonstrated by the localization of the carcinoid myomatoses), acts almost exclusively on the nerves of the mucosa and on the muscles of the muscularis mucosae?

Summary and Conclusions

1. Originating in the nerves of the mucosa, previously hypertrophied, carcinoids penetrate the myenteric plexuses progressively without destroying them and without provoking their hyperplasia. They merit the name of ***neurocarcinoid***. Invasion of the connective tissue and of the lymphatics is secondary.

2. Connective tissue infiltrated by carcinoids does not originate muscle fibers. On the contrary, when the nerves are invaded by carcinoids, the corresponding muscle coats undergo hyperplasia restricted to the territory of the infected nerve. The muscle fibers formed in the interstices of carcinoids, then, are not an integral part of these tumors; they result from proliferation of preëxisting muscles provoked by the presence of argentaffin cells in their nerves.

3. This myogenic action of the argentaffin cells seems due to a product of limited diffusability secreted by the cells into the nerves (neurocrinia).

4. As a working hypothesis, one may suppose that the normal argentaffin cells of the intestinal mucosa function like the cells of carcinoids, and that their secretion poured into the plexus of the mucosa plays a rôle in the functioning of the muscularis mucosae.

I must again thank my friend, Dr. Laidlaw, for his translation of this paper for the Journal.

REFERENCES

1. Oberndorfer, S. Die Geschwülste des Darmes. Handbuch der speziellen pathologischen Anatomie und Histologie. Henke, F., and Lubarsch, O. Berlin, 1929, **4**, part 3, 814.

2. Masson, P. Contribution to the study of the sympathetic nerves of the appendix. The musculonervous complex of the submucosa. *Am. J. Path.*, 1930, **6**, 217.

3. Masson, P., and Berger, L. Sur un nouveau mode de sécrétion interne: la neurocrinie. *Compt. rend. Acad. d. sc.*, 1923, **176**, 1748.

4. Ehrlich, S. L. The origin of carcinoids of the appendix. Carcinoides of the intestinal tract and immature neuromas. Neurocytomes. *Kharkow méd. Bull.*, 1914.

5. Masson, P. Carcinoids (argentaffin cell tumors) and nerve hyperplasia of the appendicular mucosa. *Am. J. Path.*, 1928, **4**, 181.

6. Danisch, F. Zur Histogenese der sogenannten Appendixcarcinoide. *Beitr. z. path. Anat. u. z. allg. Pathol.*, 1923–24, **72**, 687.

7. Ciaccio, C. Sur une nouvelle espèce cellulaire dans les glandes de Lieberkühn. *Compt. rend. Soc. de biol.*, 1906, **60**, 76.

DESCRIPTION OF PLATES

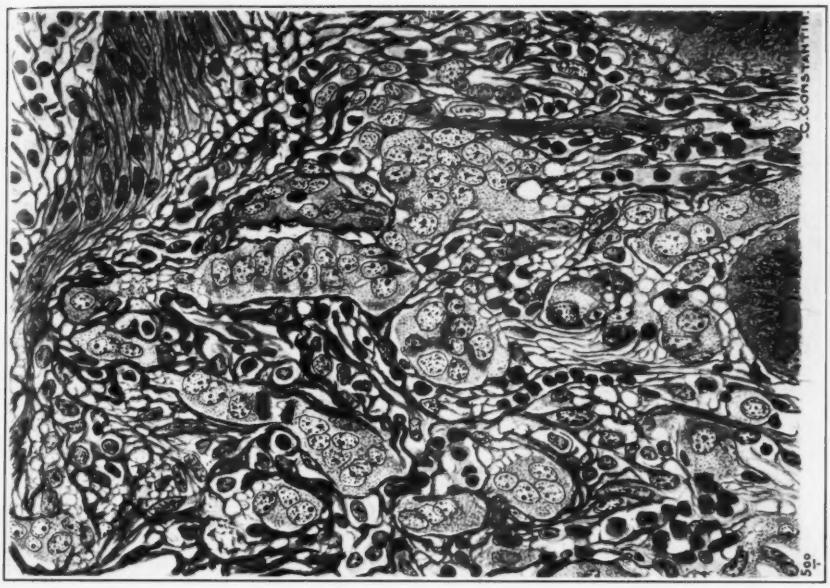
PLATE 103

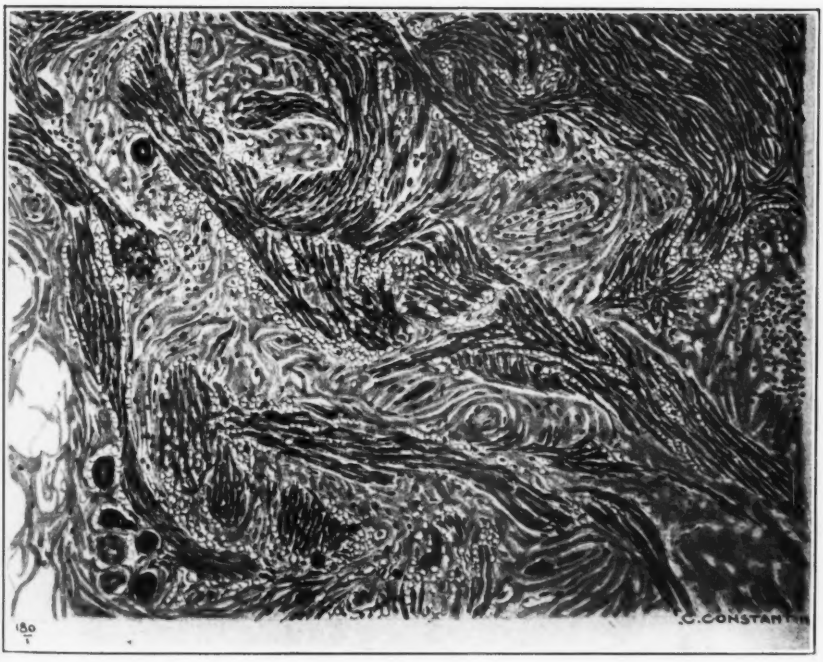
FIG. 1. Obs. 3 (123 m). Deep region of the mucosa.

On the left the muscularis mucosae "M." On the right, the tips of two crypts of Lieberkühn "LL." Between the muscularis mucosae and the tips of the crypts, lymphoid tissue and slender columns of carcinoid cells. These cells are not situated in the lymphoid tissue but in the filaments of the periglandular and subglandular plexuses, which they fill incompletely. The regions "N" where the nerves are not completely infiltrated show (1) that the collagenous sheath around the carcinoid column is none other than the sheath of the invaded nerve; and (2) that the nerve segments visible between two infested regions have undergone no degeneration. The continuity of the nerves, then, has not been broken by the invading cells.

FIG. 2. Obs. 3 (123 m). Middle region of the submucosa. The appendicular mucosa is on the right and above, the peritoneum on the left and below.

The anastomosing muscle bundles (dark gray) correspond to the cells of the muscular mechanism of the submucosa, locally hypertrophied. In the intervals, there is fibrous tissue (pale gray). The black spots represent carcinoid columns penetrating Meissner's plexus. None of them lies directly in the connective tissue. The muscle hyperplasia is limited strictly to the zone of infiltration of the nerves.





Masson Muscular "Stroma" of Argentaffin Tumors

Plate 104

Fig. 3. Obs. 3 (123 m). Middle region of the myoma developed at the expense of the circular muscle.

The branches of the nerve plexus are injected by argentaffin cells, making them more prominent. The invasion does not involve the whole plexus but leaves free some of the branches "N," the slender ones. The cells, their contours indicated on the tracing by the dotted line, are ganglion cells of Auerbach's plexus. Despite the accumulation of argentaffin cells around them, these cells are perfectly intact. In the meshes of the plexus, there are many young muscle cells without definite orientation, probably arising from the circular fibers. On the right, lymphoid infiltration, the residue of recent inflammation.

Fig. 4. Obs. 3 (123 m). Region adjoining Fig. 3.

Node of Auerbach's plexus invaded by argentaffin cells. Three slender nerve branches "n" detach themselves. This node has no ganglion cells.

Observe the lack of orientation of the muscle fibers and the frequent presence of several nuclei in the same cytoplasmic spindle. The cells seem to multiply amitotically exclusively.

3

4

METASTASIZING "CARCINOID" TUMOR OF JEJUNUM *

István Gáspár, M. D.

(From the Laboratory of the Rochester General Hospital, Rochester, N. Y.)

Carcinoids are usually multiple tumors occurring in the small intestine, appendix, and occasionally in the colon. Histologically they are formed of epithelial cells arranged in communicating strands, irregularly sized and embedded in stroma. Lubarsch [1] in 1888 reported the first cases as primary carcinomas of the ileum. In 1907 Oberndorfer [2] applied the name "carcinoid" to these tumors. Although only about seventy cases comprise the total number of carcinoid of the small intestine, a considerable literature has dealt with their case reports, etiology, histopathology, physiology and ultimate prognosis. Before Masson's silver impregnation method of staining, most investigations followed purely morphological lines. By the silver method Lubarsch was able to demonstrate a definite relationship between the nests and columns of tumor cells with those in the crypts of Lieberkühn, thus suggesting the epithelial origin of these growths. At the same time he pointed out the distinct difference between these cells and those of adenocarcinomatous tumors occurring in other parts of the gastro-intestinal tract. The opinion of Lubarsch was shared by Bunting, [3] Burckhardt, [4] Versé, [5] and Krompecher,[6] while Saltykow,[7] Oberndorfer,[2] and Toenniessen [8] failed to demonstrate any evidence in support of the intestinal epithelial origin of these cells. Because of their structural similarity to the islands of Langerhans and their multiple occurrence, Saltykow held them to be derived from misplaced embryonic islands of Langerhans. Oberndorfer, on the other hand, considered them embryonal malformations, while Toenniessen asserted that they were misplaced, heterotopic and spread-out intestinal epithelium. By means of the silver impregnation method Masson [9] found that besides being chromaffin in nature, as was pointed out by Oberndorfer, the cells of the carcinoid tumors reduced solutions of ammoniacal silver in the same manner as the so-called Nicholas-Kulchitzky-Schmidt cells of the intestinal epithelium. Hence he called the carcinoids "argentaffin cell tumors." His contention has been confirmed by Hase-

* Received for publication May 9, 1930.

gawa,[10] Danisch,[11] Forbus,[12] and Hamperl.[13] Hasegawa has shown, however, that the silver impregnation method has occasionally been irregular in tumors having the identical histological picture. One may say then that the silver impregnation method has shed much new light on these cells in their relationship to the cells in the crypts of Lieberkühn, although opinions still differ concerning the true nature of these tumors. The question of origin and malignancy remains unsettled, though several instances of metastasis have already been reported.

This report deals with a new case of carcinoid tumor of the small intestine with metastasis, observed at the Rochester General Hospital, which, it is hoped, may shed some light on the pathology and clinical significance of these tumors.

Report of Case

Clinical History: A. F. F. (Hospital No. 50909), white, female, 74 years of age, admitted on April 15, 1929, had had frequent indigestion and anorexia over a long period. She had no trouble on the prescribed diet. In March, 1929, a constant pain developed in the back, and since that time the patient was confined to bed. She suffered from constipation. Physical examination was essentially negative. Some tenderness was noted, especially on the left side and in the epigastrium. The liver was palpable one finger's breadth below the costal margin. Roentgenograms showed a dilated small bowel. The patient died on April 23, 1929.

Clinical Diagnosis: Possible malignancy of gastro-intestinal tract, or head of pancreas.

Postmortem Examination

In the lower jejunum a tumor measuring 15 by 13 by 12 mm. was found situated in the wall directly opposite the mesenteric insertion (Fig. 1). The tumor bulged toward the lumen of the jejunum producing an almost complete obstruction. Only the tip of the enterotome was admitted through the lumen. The jejunum was much dilated above, and collapsed below, the tumor mass. In gross sections the mucosa and intestinal muscular layers were found to be infiltrated by the neoplasm. In this area the width of the intestinal wall measured from 5 to 10 mm. The serous layer was drawn into a "V" shape by the tumor and the opposite serous surfaces were adherent. In the mesentery, adjoining this region, a hemorrhagic tumor the size of a hen's egg was found. Hemorrhages were observed in the surrounding mesenteric fatty tissue, as well as in the peritoneum. The liver, corset-type in appearance, was slightly

atrophied. On its surface a few grayish white, firm foci were noted which measured 6 to 8 mm. in diameter. These foci corresponded in situation to the metastatic tumors immediately below the liver capsule. On section a few smaller nodules of metastatic tumors were found deeply embedded in the liver. About 15 cm. below the above described jejunal tumor was a smaller tumor, measuring 3 by 4 by 4 mm., and 10 cm. below this mass another tumor 2.5 by 2 by 2 mm. in size was found in the submucosa opposite the insertion of the mesentery.

The autopsy revealed further a generalized arteriosclerosis with arteriosclerotic kidneys, brown atrophy of the heart, and slight pulmonary edema. The cause of death was given as carcinoma of the small intestine, with intestinal obstruction.

Histological Examination

Large Tumor Nodule: Numerous serial sections, 5 or 6 microns thick, were prepared from several blocks. A considerably thickened mucosa surrounded the tumor. Centrally, the tumor contained an ulcerated area 2 by 3 mm. in diameter, which extended to the muscularis mucosae layer. The peripheral portion of mucosa overlying the tumor appeared normal or slightly compressed. The papillae of the mucosa were distinctly seen in the peripheral portion as well as in the central parts, although centrally they seemed compressed, flattened, and not clear-cut, indicating superficial necrobiosis. Approximately one-third of the surface area of the mucosa covering the tumor showed definite thickening, which corresponded to the middle third of the tumor mass. Sections from this area revealed glands of Lieberkühn in the superficial layers of the mucosa. The mucosa below these glands was occupied by a great number of tumor cell nests. In general, the glands failed to show signs of compression, although a few such compressed glands did occur. Wherever the columns of tumor cells were scarce, the glands of Lieberkühn occurred with regularity in the deeper parts of the mucosa. Certain changes were noted in the glands of Lieberkühn not usually observed in normal glands, and the cells, instead of occurring in one row, were irregularly arranged. Among these were found many smaller cells possessing each a darkly stained nucleus. These cells preponderated in the basal portions of the glands and seemed characteristically identical with the tumor cells occurring outside the glands of Lieber-

kühn (Figs. 2 and 3). Again, these tumor cells showed a tendency to form branches interconnecting the many tumor masses and all traceable to the same gland of Lieberkühn. Fig. 4 demonstrates how sometimes these tumor cells would fill the intravillous spaces, thus coming in direct contact with the intestinal lumen. Frequently the tumor cells would penetrate into the submucosa, forming in this layer much larger masses, covered toward the periphery by unaltered mucosa in which no tumor cells were found. Two hundred serial sections, each about 5 microns in thickness, prepared from the edge of the tumor, failed consistently to show any tumor cells in the mucosa. The circular and longitudinal layers of muscular fibers, however, as well as the serosa, were well infiltrated by the tumor cells, which assumed the shape of thin bundles in the muscular layers, and nodular forms as large as 4 to 5 mm. in diameter, in the serosa.

The stroma of the tumor was made up of loose connective tissue, hyalinized in many areas. Smooth muscle cells could be found in the vicinity of blood vessels. Moderate infiltration of round cells was present in the upper half and in the ulcerated parts of overlying mucosa. Few blood vessels occurred within the tumor, but toward the periphery they became more numerous and more dilated. Tumor cells were found within the blood vessel lumina (Fig. 5).

Sections made from the metastatic nodule in the mesentery showed a large central necrotic area. This was surrounded by fibrosis and blood vessels, while toward the periphery a wide layer of tumor cell masses was embedded in a stroma of hyaline connective tissue. In this area numerous blood-filled sinuses occurred (Fig. 6). The sinus walls had no endothelium and seemed formed entirely of tumor cells. In this area also, the blood vessels were invaded by masses of tumor cells.

The lymph node in the neighborhood of the mesenteric tumor nodule showed masses of metastatic tumor cells. Especially toward the periphery, the dilated blood and lymph vessels were filled with tumor cells (Fig. 7). These vessels retained their endothelial lining.

Sections of a cherry-sized metastatic tumor nodule in the liver showed central necrosis, while at the periphery were found numerous large and freely anastomosing masses of tumor cells (Figs. 8 and 9). The blood vessels, which occurred mostly at the periphery, contained masses of tumor cells.

The morphology of the encountered tumor cells agreed in the main with that described by numerous investigators, and in particular with Masson's excellently described studies. Thus the cells appeared round or polygonal, palisade, columnar or prismatic, all varieties of the same species according to Masson. Close to their origin in the glands of Lieberkühn, the round cell type dominated. The palisade cells, inconstantly present, occurred in the irregular columns of tumor masses. The prismatic cells were found around small cavities which sometimes contained small amounts of homogeneous material. The nuclei of the tumor cells varied between round, oval or spindle-shaped forms, all staining very deeply. Mitotic figures were not observed, although carefully sought for. Sections stained by the hematoxylin-eosin method showed the cytoplasm of the tumor cells to be filled by minute acidophilic granules — chromaffin and argentaffin granules. The sections were stained by Masson's and the blocks by Hasegawa's silver impregnation methods. Positive results were obtained by both methods. The sections stained by Masson's method showed the silver-reduced granules as dark brown in color and exceedingly fine. This method preserved the entire structure very well and produced excellent demonstrating material.

The tumor cells in the metastatic nodules were identical with those found in the primary tumor and yielded positive results by the silver impregnation method. The tumor cells in the metastatic areas in the liver seemed slightly larger, the nuclei more uniformly round or vesicular, a condition perhaps explainable by a somewhat reduced pressure. Complete absence of mitotic figures was noted in these cells.

Four hundred serial sections were prepared from the second and third tumor nodules found in the jejunum. Because of superficial necrobiosis, the second tumor was found unsuitable for etiological study. The overlying mucosa seemed thicker at the tip of the nodule, where columns of round or elongated tumor cells occurred. Longitudinal or cross strands of crypts of Lieberkühn could be seen. Occasionally a column of tumor cells was located between the villi, thus coming into direct contact with the intestinal lumen. The overlying mucosa near the tip was thinned out, but further on regained its normal appearance. The muscularis mucosae was invaded by tumor cells in many places. The bulk of the tumor oc-

curred in the submucosa where it occupied a much wider area than in the mucosa. The muscular fibers likewise showed infiltration by masses of tumor cells, presenting the same features as already described in the first tumor, although hyalinization of the stroma was not marked.

The small tumor of the jejunum, which measured 2.5 by 2 by 2 mm., presented a much thickened mucosa, corresponding to that of the tip. Immediately adjoining the tumor, the mucosa was somewhat thinned out, but further on it resumed its normal appearance. In the thick portion many columns and masses of tumor cells appeared. The course of the tumor columns seemed perpendicular and running in the same direction as the crypts of Lieberkühn, which were cut lengthwise. Cross-sections of the glands of Lieberkühn, which appeared in several places in this area, did not indicate any pressure. These glands occasionally occurred in direct contact with the tumor cells which had broken through the muscularis mucosae layer (Fig. 12). Fortunately, a section was made directly into a gland of Lieberkühn which emptied into the intestinal lumen; in its lower half it showed an immediate contact of the tumor cells with the apparently normal cells of the crypt (Figs. 10 and 11). In the few sections which followed, only a cross-cut of the gland was obtained, but in these sections the original column of tumor cells was found to have broken through the muscularis mucosae layer into the submucosa.

In several similarly prepared pictures, a direct contact was found to exist between the tumor cells and the glands of Lieberkühn. The bulk of the tumor rested in the submucosa, although a few cells had reached the muscularis mucosae layer. No round cell infiltration was observed in the tumor stroma in the mucosa. Numerous capillary blood vessels were found in the submucosa. No apparent difference could be detected in these cells from those occurring elsewhere. The silver impregnation method of staining was not carried out in the two smaller tumors because of their previous fixation in formalin.

Discussion

The jejunal tumors described obviously correspond with the small tumors first reported by Lubarsch and subsequently called "carcinoids" by Oberndorfer. Their occurrence in an elderly individual, their multiple nature, the sharp histological difference

from adenocarcinomas, and their histological identity with known carcinoid tumors of the small intestine, confirm their identity as such. Of greatest interest to the histopathologist is the origin of these tumor cells, their infiltrating character, and their tendency to form carcinoid metastases. Because of the disagreement among investigators about the origin of carcinoid tumors, a special attempt was made to prepare a large series of sections in order better to trace their origin and their course of metastases. It is evident from Figs. 2 and 3 that the tumor cells accumulate at the tip of the glands of Lieberkühn within the basal membrane. The tumor cells are found immediately beneath the lining cells and in intimate contact with the Lieberkühn glands. The serial sections reveal that the basal membrane is being pushed out by the tumor cells in a manner similar to the budding described by Masson in a case of appendical carcinoids. During the early stages of development, these budding forms do not appear to break through the basal membrane, and in some sections they fail to unite with columns of tumor cells. Other buds, however, have perforated the basal membrane and can be traced into the deeper layers.

It is also observed that several columns of tumor cells originate in a Lieberkühn gland and by the formation of ramifying columns the tumor steadily increases in size. It is probable that only a definite number of glands of Lieberkühn give rise to a tumor. This fact is suggested by the constant observation that columns of tumor cells occur only in the apex of the tumor nodule. The rough pyramidal shape of the tumor nodules may be explained by the abundant ramification and proliferation of the cells into the deeper layers. In a number of glands the lumina are filled with tumor cells. It is evident that the course of tumor proliferation within the glands is toward the intestinal lumen, and hence the lining cells of the glands disappear. If only slight pressure is exerted from the side by the growing tumor mass, a direct opening into the intestinal lumen may be found. If, on the other hand, pressure is brought to bear from below and from the side, the villi become compressed and their openings disappear. For this very reason it is essential to make serial sections in order to find convincing evidence in support of the origin of these tumor cells from the glands of Lieberkühn. It is probable that some of the perpendicular columns of tumor cells found in the intestinal mucosa occupied the place of the glands of Lieberkühn. No clear-cut proof of this contention is possible, how-

ever, because of the already mentioned disappearance of the lining cells.

Mention has been made in literature that the mucosa seemed to be intact especially above smaller carcinoids. From the reports we are unable to ascertain whether or not serial sections were made of these small tumors. In our case, we were fortunate to demonstrate the retention of intestinal mucosa in the apices of the tumor masses. Sections made towards the periphery, however, revealed the presence of tumor cells only in the submucosal and muscularis layers.

By means of the silver impregnation methods suggested by Masson and Hasegawa, we feel that our findings further substantiate the hypothesis concerning the glands of Lieberkühn origin of these argentaffin tumor cells; the so-called Nicholas-Kulchitzky-Masson cells normally contain the argentaffin, silver-reducing granules. It seems, therefore, quite proper to call the carcinoids by the name "argentaffin cell tumors." We believe, however, that the appellation "carcinoid" is more suitable in consideration of the tendency of these tumor cells to infiltrate and eventually to become malignant. Even the smallest nests of these cells possessed ability to infiltrate the muscular layers. On this basis our opinion is in complete agreement with those who hold that carcinoids should be classified as true carcinomas. Their proliferation is slow, but the formation of metastases is only a question of time. Metastases take place mainly through the blood stream, although occasionally dissemination may occur by means of the lymph vessels. We have already demonstrated the abundant presence of tumor cells along the walls of the dilated blood vessels and the gross hemorrhages which gave the characteristic hemorrhagic appearance to the large metastatic tumor found in the mesentery. Versé also reported metastases both through blood and lymph vessels, for in two cases he noted tumor cells in the mesenteric veins, and in both the blood and lymph vessels of a mesenteric lymph gland. Burckhardt also found small groups of tumor cells among the blood cells in a mesenteric vein.

Metastasizing carcinoids of the small intestine have been described by Ransom,[14] Versé,[5] and Schopper.[15] The case reported in this paper should be added to these already recognized metastatic carcinoids.

Very little can be said about the actual etiology. Several investigators found chronic inflammatory processes in the intestinal tract associated with carcinoids. It is possible that a direct relationship

exists between chronic irritation and carcinoids, although we failed to demonstrate any round cell infiltration in the mucosa adjoining the smallest tumors in our case. The invasion of round cells into the mucosal part of the large tumor seemed secondarily to be due to ulceration.

Saltykow believes that carcinoid tumors are of no clinical importance and are only accidentally discovered at autopsy. His conclusion seems unwarranted, inasmuch as the seventh case reported by him presented a carcinoid tumor in the ileum, measuring 2.5 by 2.5 by 1.5 cm., occurring 6 cm. above the ileocecal valve and causing almost complete obstruction, with corresponding clinical symptoms. Judging from the history of his case, this tumor was the actual cause of the patient's death. In our case, the tumor, which measured 15 by 13 by 12 mm., had probably caused intestinal disturbance for some time, until it finally brought about almost complete obstruction. Roentgenograms had shown dilated loops of the small intestine, and at autopsy the carcinoid tumor was found in the position indicated by the films. It is certain that had the patient sought hospital care earlier, resection of the involved part of the jejunum might have saved the patient's life and prevented metastases. It appears, therefore, that carcinoid tumors may occasionally assume clinical significance.

Summary

1. A case of multiple carcinoid tumors of the jejunum, one of which caused intestinal obstruction, with metastases in the mesentery and liver, is reported.

2. Histological examination revealed the picture of typical carcinoid tumors. Serial sections definitely indicate that at least two of the tumors originated in the crypts of Lieberkühn.

3. Positive silver impregnation of the tumor cells confirms Masson's contention that the origin of carcinoid tumors is in the Nicholas-Kulchitzky-Masson cells in the glands of Lieberkühn.

4. Carcinoid tumors may assume clinical significance.

REFERENCES

1. Lubarsch, O. Ueber den primären Krebs des Ileum nebst Bemerkungen über das gleichzeitige Vorkommen von Krebs und Tuberkulose. *Virchows Arch. f. path. Anat.*, 1888, **111**, 280.
2. Oberndorfer, S. Karzinoide Tumoren des Dünndarmes. *Frankfurt. Ztschr. f. Path.*, 1907, **1**, 426.

3. Bunting, C. H. Multiple primary carcinomata of the ileum. *Bull. Johns Hopkins Hosp.*, 1904, **15**, 389.
4. Burckhardt, J. L. Zur Lehre der kleinen Dünndarmkarzinome. *Frankfurt. Ztschr. f. Path.*, 1909, **3**, 593.
5. Versé. Ueber die Histogenese der Schleimhautcarcinome. *Verhandl. d. deutsch. path. Gesellsch.*, 1908, **12**, 95.
6. Krompecher, E. Ueber die Basalzellentumoren der Zylinderepithelschleimhäute mit besonderer Berücksichtigung der "Karzinoide" des Darms. *Beitr. z. path. Anat. u. z. allg. Pathol.*, 1919, **65**, 79.
7. Saltykow, S. Ueber die Genese der "karzinoiden Tumoren," sowie der "Adenomyome" des Darmes. *Beitr. z. path. Anat. u. z. allg. Pathol.*, 1912, **54**, 559.
8. Toenniessen, E. Untersuchungen über die in der Submukose des Dünndarms vorkommenden epithelialen Tumoren. *Ztschr. f. Krebsforsch.*, 1909–10, **8**, 355.
9. Masson, P. Carcinoids (argentaffin-cell tumors) and nerve hyperplasia of the appendicular mucosa. *Am. J. Path.*, 1928, **4**, 181.
10. Hasegawa, T. Ueber die Carcinoide des Wurmfortsatzes und des Dünndarmes. *Virchows Arch. f. path. Anat.*, 1923, **244**, 8.
11. Danisch, F. Zur Histogenese der sogenannten Appendixkarzinoide. *Beitr. z. path. Anat. u. z. allg. Pathol.*, 1923–24, **72**, 687.
12. Forbus, W. D. Argentaffine tumors of the appendix and small intestine. *Bull. Johns Hopkins Hosp.*, 1925, **37**, 130.
13. Hamperl, H. Ueber die "gelbem (chromaffinen)" Zellen im gesunden und kranken Magendarmschlauch. *Virchows Arch. f. path. Anat.*, 1927, **266**, 509.
14. Ransom, W. B. A case of primary carcinoma of the ileum. *Lancet*, 1890, **2**, 1020.
15. Schopper, K. I. Ueber ein "kleines Dünndarmkarzinom" mit ausgedehnter Metastasenbildung. *Verhandl d. deutsch. path. Gesellsch.*, 1913, **16**, 387.

DESCRIPTION OF PLATES

PLATE 105

FIG. 1. Carcinoid tumor 15 by 13 by 12 mm. in size, which caused intestinal obstruction and metastases in the mesentery and liver.

FIG. 2. Lieberkühn gland in cross-section from the large jejunal tumor. A few tumor cells lie beneath the lining cells of the gland. They form a definite group within the pushed-out limiting membrane.

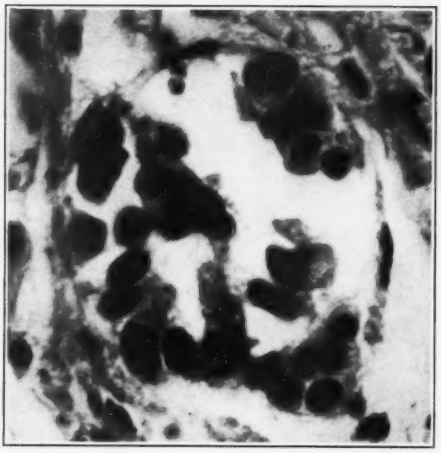
FIG. 3. The same gland as in Fig. 2 as it appears in the following serial section. The tumor cell group is somewhat larger.

FIG. 4. Tumor cells filling the space between villi of a Lieberkühn gland.

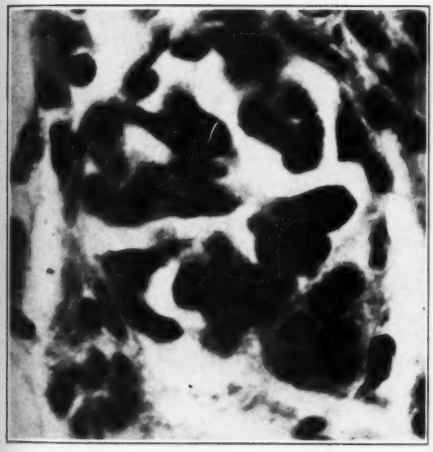
FIG. 5. Large tumor cell group in a blood vessel of the carcinoid tumor of the jejunum, 15 by 13 by 12 mm. in size.

FIG. 6. A section from the metastatic nodule in the mesentery. Numerous wide sinuses filled with blood and surrounded by tumor cells.

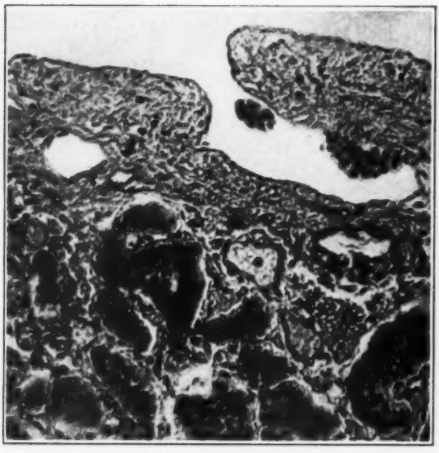
1



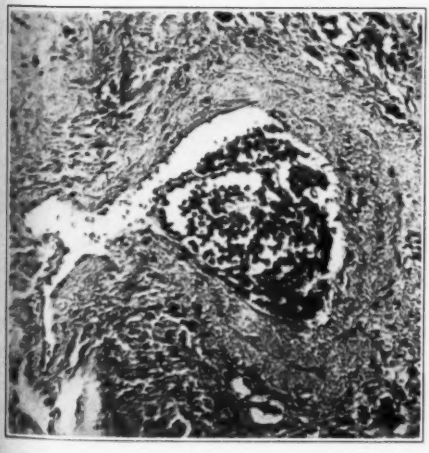
2



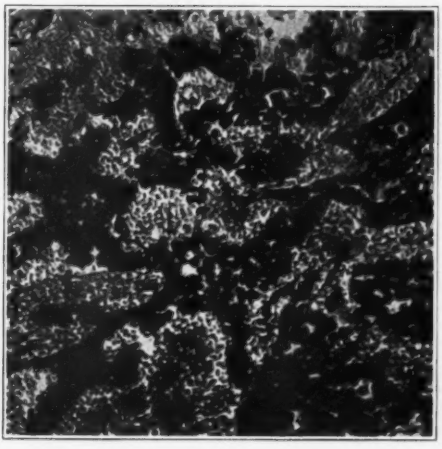
3



4



5



6

Gáspár

Metastasizing "Carcinoid" Tumor of Jejunum

PLATE 106

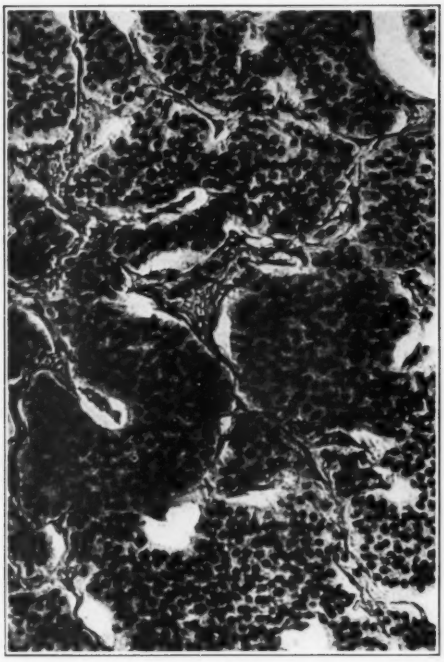
FIG. 7. Metastatic tumor cell groups in the lymph sinuses of a mesenteric lymph gland.

FIG. 8. Section from a metastatic nodule in the liver.

FIG. 9. The extreme periphery of a metastatic liver tumor nodule.

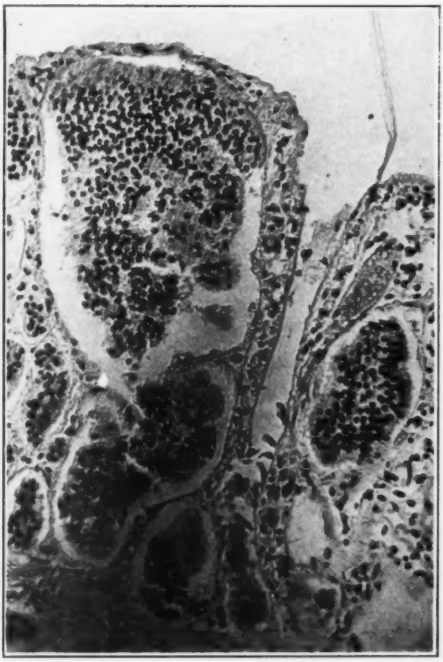
FIG. 10. Section from the smallest carcinoid tumor of the jejunum. On the right side a Lieberkühn gland is cut longitudinally and empties into the lumen of the intestine. A tumor bundle originates in the gland and fills out a part of the gland lumen.

7



8

9



10

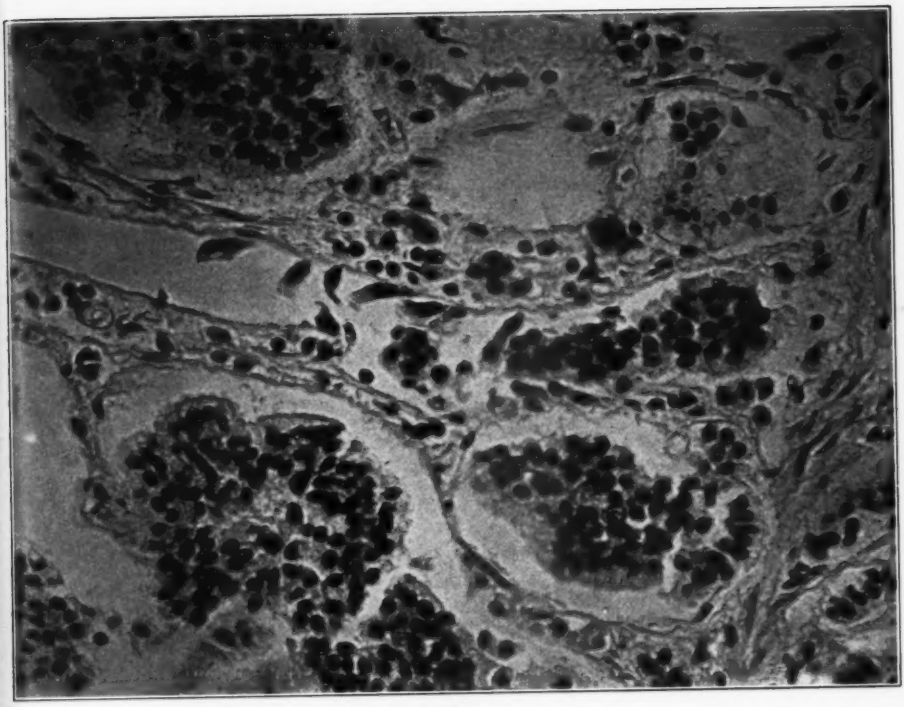
Gáspár

Metastasizing "Carcinoid" Tumors of Jejunum

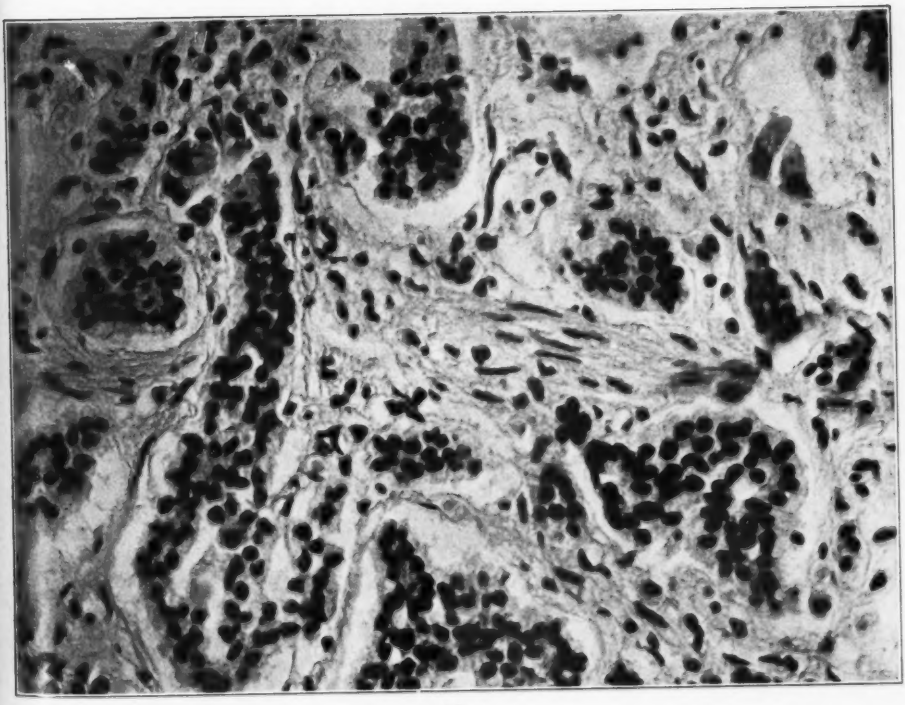
PLATE 107

FIG. 11. Higher magnification of Fig. 9. The origin of the tumor cells from the Lieberkühn gland is quite convincing. The tumor cells are in immediate contact with the cells in the crypt and fill its lower portion.

FIG. 12. Section from the smallest jejunal tumor. Above the mucosae muscularis two Lieberkühn glands are cut across. On the left side, the Lieberkühn gland gives rise to a column of tumor cells which breaks through the mucosae muscularis. Branching of the same column can be made out.



11



12

Gáspár Metastasizing "Carcinoid" Tumor of Jejunum

RETICULUM. ITS ORIGIN. THE OCCURRENCE OF RETICULUM FIBRILS IN CAPILLARY ENDOTHELIUM. A NEW METHOD OF DEMONSTRATION. II. THE FINER CAPILLARY BED *

JAMES F. RINEHART, M.D.

(From the Department of Pathology, University of California Medical School, San Francisco, California)

The present report is the first of a series of studies dealing with the derivation and differentiation of supportive substances from the mesenchyme as revealed by a new method of metallic impregnation described in detail below. Particular attention is devoted to the origin of reticulum and its occurrence in the mature organism. Other phases of mesenchymal differentiation are reserved for further study. In a preliminary way, fully realizing factors of incompleteness, a new concept of the finer capillary bed is presented.

A considerable literature has accumulated upon the subject of reticulum since Mall [1] in 1891 announced his discovery that the framework of many organs and tissues of the mammalian body is composed neither of white fibrous connective tissue nor of yellow elastic tissue, but of a third type of supporting substance composed of fine interlacing fibrils which not only differ from the white fibrous tissue in appearance, but are more resistant to acid and alkaline solvents, and are not so readily attacked by digestive ferments.

The introduction of the Bielschowsky method of silver impregnation stimulated renewed interest in reticulum. No attempt will be made in the present paper to review the rather extensive literature upon this subject. Some idea of its unsettled status may be gained by citing the contrasting concepts of Corner [2] (1920), and Mallory and Parker [3] (1927). Corner described reticulum fibrils arising in and extending from the capillary endothelial cells of the corpus luteum, adrenal, hypophysis and kidney. This author is one of the few making the distinct assertion that capillary endothelial cells in the adult organism produce a fibrillar substance, and identifying this substance with reticulum because of its affinity for impregnation with silver by the Bielschowsky method. Opposed to this view is

* Received for publication May 15, 1930.

that expressed by Mallory and Parker who deny formation of reticular fibrils by ordinary capillary endothelium, and assert that it is produced only by fibroblasts. The black impregnated fibrils are said to be fibrils originating in fibroblasts taking a black impregnation reaction due to a fine dispersion of the fibrillar substance.

Most of the other contributions deal with the occurrence of reticulum in various organs and tissues under normal and pathological conditions, without positive statement of its derivation.

Without further reference at this time to the literature, the method we have employed, our findings and their interpretation will be set forth.

The Methods Employed

Kinney [4] (1928) briefly reported the observation, that, in tissues fixed in a solution of 4 per cent formaldehyde containing 1 per cent sodium sulphantimonate, a substance was impregnated which she considered to be reticulum. This fixation was applied to tissues by the writer and a large variety of counterstaining methods applied. Results gave sufficient encouragement to pursue the subject further. Finally a silver ammonium carbonate solution was employed in tissues so fixed and a surprisingly sharp impregnation of reticulum was observed. Various refinements were devised. The following methods of fixation, impregnation and counterstaining were adopted which yielded the most satisfactory sections. These methods were employed in this study.

Fixation: Primary fixation in 4 per cent formaldehyde, preferably neutral, or Kaiserling's solution No. 1 is used. Following this, tissues are treated essentially as in the Kaiserling method of preservation for museum specimens. The tissues are washed in running water 24 hours, passed into 80 per cent alcohol for 24 hours, and then into Kaiserling's solution No. 3 for 3 or more days. Satisfactory preparations may be obtained in sections that have been long preserved in formalin or in Kaiserling's solution No. 3. Sections are taken from the Kaiserling solution and washed in running water for 12 to 24 hours. After washing, sections cut properly for paraffin blocks, 2 to 3 mm. in diameter, are mordanted or refixed in a freshly prepared 0.5 per cent solution of sodium sulphantimonate made in a 4 per cent solution of neutral formaldehyde. This refixation is employed for 24 to 48 hours. After this mordanting, sections are again washed

in running tap water for 24 hours, then passed into 80 per cent alcohol, dehydrated in the usual manner by running through 95 per cent and absolute alcohol, cleared in xylol and embedded in paraffin. Sections are cut the desired thickness and impregnated in the following manner.

Impregnation: Two methods of impregnation may be used, one involving gold toning, the other not. Both methods are detailed below. Either method gives satisfactory results. Toning transforms the golden brown reaction of collagen in silver sections into a rose red.

The silver impregnation method is essentially that devised by Foot for paraffin sections and detailed in McClung's "Microscopic Technique." [5] All preliminary treatment with sodium hyposulphite, potassium permanganate and oxalic acid, are, however, omitted. The gold toning employs a modification advised by Laidlaw [6] in the use of oxalic acid after the gold chloride toning bath.

Method I. Without Gold Toning

1. Remove paraffin and place sections in water in usual manner.
2. Impregnate with silver ammonium carbonate solution (prepared as described in footnote *) for 30 minutes in oven at 37° C.
3. Wash sections in distilled water — about 8 to 10 consecutive changes.
4. Immediately pour on 4 per cent *neutral* formladehyde and let stand for 5 minutes.
5. Rinse sections in distilled water several times.
6. Fix in 5 per cent sodium hyposulphite 2 to 5 minutes.
7. Wash at tap for 4 or more hours.

* *To Prepare Silver Ammonium Carbonate.*

1. Mix 12 cc. each of 10 per cent solution of silver nitrate and a saturated solution of lithium carbonate.
2. Wash precipitate 5 or 6 times with about 75 cc. of distilled water.
3. Add about 50 cc. of distilled water to washed precipitate and dissolve precipitate by adding ammonium hydroxide drop by drop until solution is almost clear. (Do not add too much — leave a few granules undissolved.) About 12 to 15 drops of ammonium hydroxide are needed.
4. Add distilled water to make solution of 85 to 100 cc. and filter.

Note: Solution must be prepared fresh for each batch and should be discarded after use. The solution is unstable and if exposed to the combined action of light and alcohol, there is danger of formation of an explosive mixture.

Staining Results: Mesenchymal fibrils range from yellow and golden brown to black. Reticulum fibers black. Collagen fibers golden brown (transition colors seen).

Method II. With Gold Toning

1. Remove paraffin from sections and place in distilled water in usual manner.
2. Impregnate with silver ammonium carbonate (freshly prepared each time and discarded) for 30 minutes in oven at 37° C.
3. Wash sections in distilled water about 8 to 10 consecutive changes.
4. Immediately pour on 4 per cent *neutral* formaldehyde, let stand for 5 minutes to reduce silver.
5. Rinse well in distilled water.
6. Tone in aqueous gold chloride solution 1:250 (acid yellow gold chloride) for 5 minutes.
7. Rinse well in distilled water.
8. Pour 5 per cent oxalic acid on sections for 5 to 8 minutes.
9. Wash well in distilled water.
10. Fix with 5 per cent aqueous solution sodium hyposulphite. Change the hyposulphite as many times as necessary until solution is clear.
11. Rinse in distilled water and wash at tap 4 to 6 hours before counterstaining.

Staining Results: Mesenchymal fibrils, delicate rose red to black. Reticulum fibers, black. Collagen fibers, rose red.

Counterstaining: Counterstaining by the May-Grünwald-Giemsa method has given the most satisfactory results. The method is essentially similar to that employed by Downey [7] in his studies of developing lymph nodes.

1. Place sections 15 minutes in dilute acetic acid (6 drops per 100 cc.).
2. Place sections 15 minutes in distilled water.
3. Stain in equal parts of saturated methyl alcohol solution of May-Grünwald eosinate of methylene blue, and distilled water 1 to 2 minutes.
4. Rinse sections in distilled water.

5. Giemsa stain diluted 1:15 for 15 minutes.
6. Wash in distilled water.
7. Differentiate in dilute acetic, 6 drops to 100 cc. for ½ to 2 minutes. (This differentiation should be watched under the microscope.)
8. Rinse in distilled water.
9. Dehydrate rapidly in acetone.
10. Clear in cedar oil, 1 minute.
11. Xylol.
12. Mount in thickened cedar oil.

A simpler, yet satisfactory counterstain, is the routine hematoxylin and eosin.

The above described methods have in the writer's hands given clean sections with clear differentiation. Connective tissue fiber substances including reticulum are completely impregnated and a satisfactory polychrome counterstain secured. In addition to applying the method to adult human tissues, both normal and pathological, the method has been employed in a small series of pig embryos of 7.5, 11 and 22 cm. This has greatly helped in achieving an understanding of reticulum in the normal fully developed human organism.

The Mesenchyme

The mesenchyme consists of cells with round, oval or slightly irregular nuclei usually surrounded by a small amount of faintly staining cytoplasm (the endoplasm) from which radiate delicate fibrils and fibers which are impregnated by the method employed. An exceptionally rich fibrillar mesh is formed, the fibrils of adjacent cells readily anastomosing with one another. These fibrils impregnated with silver alone range from a yellowish or golden brown color to black, and in toned sections from a delicate rose red to black. At nodal points where the fibers cross, the depth of the tone is increased. Mall [8] (1902) showed, that in the intestine, reticular fibrils develop in the cytoplasm of the mesenchymal syncytium and later Hueck [9] (1920) affirmed the same thing for mesenchyme in general.

An abundance of mesenchyme at this stage of differentiation is available for examination in the embryos studied. Phases of the differentiation of cartilage, fiber bone, skeletal and smooth muscle have

been traced with comparative ease using the method described. This will be the subject of a subsequent report.

Differentiation of Fibrous Tissue in the Mesenchyme: The morphological details of the differentiation of mesenchyme into fibrous tissue are so intimately associated with the problem in hand that an outline of this phase is deemed essential. As previously indicated, the delicate fiber substance of the simple mesenchyme shows shades ranging from yellow to black in untoned sections, and delicate rose to black in toned sections. With a condensation of fibrillar substance the color becomes more distinctly golden brown in simple silver prepared sections, and rich rose red if followed by gold toning (the color reactions of collagen). The transformation of simple mesenchyme into fibrous tissue entails a realignment of cells and fibrillar substance with the alteration in impregnation reaction indicated. The differentiation undoubtedly follows definite mechanical principles. For example, a bronchus enlarging in the mesenchyme acquires at its periphery a collagenous reacting mesenchyme—that is fibrous tissue. The fibers assume a radial arrangement about the expanding structures, the nuclei elongate in the direction of the fibers, the fibers now become more closely grouped, and the impregnation reaction becomes the distinct golden brown of collagen. The same phenomenon has been observed in the developing cuspid valves, in the corium and in other places where permanent fibrous tissue is being laid down. Fig. 1, a section of the skin and subcutaneous tissue of a 7.5 cm. pig embryo shows the transformation of the mesenchyme into fibrous tissue in the position of the corium. The loose underlying tissue is the fibrillated undifferentiated mesenchyme. Fig. 2 shows the same in greater detail.

The Development of Capillaries and Distal Lymphatics in the Mesenchyme: While not the primary consideration of this report, observations on the formation of distal lymphatics appear so clear-cut that brief attention will be given them at this time. Studied in the interlobular septae of the lung, these appear as simple clefts in the mesenchyme shown under low power magnification in Fig. 3. In Fig. 4 a portion of the wall of a lymphatic is shown in greater detail. The lining cells show fibrillated processes anastomosing freely with those of the surrounding mesenchyme. The nuclei flatten, paralleling the lymphatic wall and the fibers coarsen slightly by condensation.

Intimately associated with the problem under consideration is the question of the derivation of capillaries. The weight of evidence favors the concept that capillaries are formed *in situ* as a direct differentiation of the mesenchyme. Among those favoring this point of view Pulford[10] cites Reichert, Goethe, Felix, Rückert, Mollier, Maximow and Bonnet. In this country McClure,[11, 12] Reagan [13, 14] and Stockard [15] have contributed convincing evidence supporting the local origin of capillary endothelium. Hueck recently lends further support to this concept. The writer's study of pig embryo sections has convinced him that capillary endothelium is formed *in situ* by a very simple modification of the mesenchyme. This transformation is probably the simplest differentiation involved in the mesenchyme. The delicate fibers of this tissue are slightly rearranged and come to encircle the tube-like cleft in the mesenchyme. The new formed capillary is only readily recognized by its content of red blood cells. The cells lining it resemble those of the undifferentiated mesenchyme, and as a matter of fact, delicate fibrils can be traced from the lining cell into the undifferentiated mesenchyme. The cells lining the capillary possess the same delicate fibrillar processes as the mesenchyme. The fibrils stain darker, black or almost so. Reference to Fig. 4 and Fig. 2 will show this clearly. In Fig. 2 the small capillary in the mesenchyme underlying the corium shows distinctly the reticulum fibrils forming its wall. The nuclei in the primitive capillary wall are morphologically identical with those of the surrounding mesenchyme, and reticulum fibers extending from these nuclei not only surround the vascular channel but anastomose with the fibers of the surrounding mesenchymal cells. A capillary with the same cytological details remains in the fibrous corium above. Fig. 5 is a photomicrograph of subepithelial tissue showing an early phase in transformation of mesenchyme into fibrous tissue, containing two capillaries in which the fibered lining cells are recognizable.

In all capillary endothelium studied, both embryonic and adult, including practically all organs and tissues, reticulum fibrils have been demonstrable, thus confirming and extending the findings of Corner.

Demonstration of the delicate fibrils in mesenchyme serves to account for the reticulum fibrils in various tumors of mesenchymal origin as observed by Mallory and Parker. The very slight modifica-

tion of the mesenchyme involved in capillary formation leaves this cell not only as one producing reticulum, but with tremendous possibilities of differentiation, a very simple one being into fibrous tissue.

Evidence of the transformation of reticulin into collagen has been set forth by Rössle and Yoshida [16] (1909) in their studies of the reticulum of lymph nodes in normal and pathological conditions. Russakoff [17] (1909) likewise found little distinction in the chemical nature of reticulin and collagen. Miller [18] (1927) in studies of the reticulum in tuberculosis, although he did not assign a specific origin for reticulum, considered it a precollagenous type of connective tissue and observed this transformation in the healing process of tuberculosis. Foot,[19] in his excellent critical review of the endothelial phagocyte, gives an extensive bibliography touching many phases of the problem in hand and cites other evidence, including his own, dealing with the transformation of reticulin into collagen. The writer too, has observed the apparent transformation of reticulum fibers into fibers staining as collagen, under a variety of circumstances. Although a consideration of the rôle of reticulum in tuberculosis, as revealed by the present method of staining, will be the subject of a future report, some of the most convincing evidence of this change has been observed in this disease. In Fig. 6 is shown a tubercle in the liver in a case of miliary tuberculosis. At the periphery of the tubercle, it may be seen that the reticulum fibers lining the liver sinusoids have become thickened; with this thickening the fibers take the golden brown color of collagen in silver preparations and the rose red in gold toned sections. Other evidences of transformation of reticulin into collagen have been observed in tuberculosis.

Reticulum in the Liver, Spleen and Lymph Nodes

The liver, spleen and lymph nodes form a special phase of the reticulum problem. Each has received considerable attention in the literature and the writer can only touch upon his findings. In the liver Kupffer [20] (1876) described the reticulum under the name of *Gitterfasern*. In well prepared sections, the reticulum fibrils of the liver are striking objects. Deep brown to jet black fibers lie against the columns of liver cells forming the immediate lining of the sinusoidal vascular channels. It is not uncommon to see fibers cross over from one side to the other. The fibers are frequently seen in

intimate association with, and radiating from cell nuclei projecting into the lumen of the sinusoid, that are considered to be the Kupffer cells. Fig. 6 illustrating the transformation of reticulin into collagen about a tubercle also illustrates the general cytology of reticulum in the liver.

From incomplete studies thus far made the writer feels that the pathology and pathological physiology of the spleen is to a large extent bound up with the problem of the fiber substance of the spleen. In spleens that can be considered essentially normal, reticulum fibrils are readily demonstrated in the capillary endothelium and in a portion of the intersinusoidal or so-called reticulum cells. Normally these are delicate fibrils and not over abundant. In Banti's splenomegaly the reticulum fibrils of the intersinusoidal cells are remarkably increased in number, coarsened, and many fibers transformed into a collagen-reacting substance, giving a golden brown silver reaction, and a rose red in toned sections. Fig. 7 illustrates such a spleen. In lymph nodes, reticulum is again demonstrable in the capillary endothelium. Rössle and Yoshida found so-called reticulum, stainable by the Bielschowsky-Maresch method, in the lymph sinuses, the lymphoid tissue and the capillaries. It was considered as a precollagenous substance and transitions into collagen were noted by them. They found no distinction between the resting reticulum cells and the so-called endothelium of the lymphoid sinuses. The writer's findings are in accord with the above and with the careful studies of Downey who derived the reticulum cells supporting the pulp cords and nodules, as well as the cells lining the lymphoid sinuses, directly from the mesenchyme, and demonstrated the capacity of both to produce reticulum fibrils. Fig. 8 shows clearly reticulum fibers extending from the endothelial cells of the lymphoid sinuses. Morphologically similar cells form the supportive stroma of the lymph cords. In other areas reticulum fibers are seen in the capillary endothelium.

Universal Occurrence of Reticulum Fibers in Capillary Endothelium

As examination of the accompanying photomicrographs and drawings indicates, fiber substance, reticulum, is demonstrable in all capillary endothelium. It would be beyond the scope of this paper

or the ability of the writer to trace the capillary formation in all organs, but an outline of two general concepts, it is believed, will explain the principles of capillary formation. In tissue directly differentiated in the mesenchyme, as the heart and skeletal muscle, undifferentiated mesenchymal cells remain lying against the differentiated structures, and being applied to adjacent fibers, a potential if not actual channel lined by fibrillated mesenchymal, now endothelial, cells exists. In epithelial structures such as the lung, thyroid and hypophysis, the epithelial cells grow into the mesenchyme leaving mesenchymal cells applied against the epithelial cell columns or alveoli, forming the lining of the finer vascular spaces. As previously indicated, Corner has shown reticulum fibrils in the capillary endothelium of the corpus luteum, adrenal, hypophysis, thyroid and kidney. Except the corpus luteum, which has not been studied, the writer has been able to confirm these findings. Fig. 9 shows an area of lesser reaction in the lung in a case of miliary tuberculosis. Here may be seen cells with distinct black fibers forming the immediate lining of capillary vascular channels. Fig. 10 shows the continuity of reticulum fibers and endothelial cells in the medulla of the adrenal. The same findings hold for the cortical endothelium shown in Fig. 11.

Fig. 12 shows the black fiber lining of the capillary endothelium of the anterior lobe of the hypophysis.

Fig. 13 shows the same for the thyroid. A single cell layer existing between the vascular channel and the epithelium precludes the possibility of this being a fibroblastic layer. The reticulum fibers are flattened down, due to the pressure of the contained colloid in the alveoli.

Fig. 14 shows two small capillaries in the brain, one containing red cells, the other smaller and empty. Here in vessels a single cell layer in thickness, are clearly shown reticulum fibrils in the endothelium. With complete impregnation of the fiber substance of the capillaries, combined with adequate counterstaining a more complete concept of the finer vascular channels has been achieved which is set forth below.

The Finer Capillary Bed: Fig. 15 shows a congested area in the cortex of the kidney of a child. Examination of this figure, which is a faithful reproduction of an actual microscopic field, readily shows the following: (1) continuity of delicate black fibers (reticulum

fibers) with the endothelial cells in a perfectly simple one cell layer; (2) the identity of the basement membrane with the above described fibers; and (3) in oval, round or angular spaces are red blood cells, *i. e.*, capillaries as seen in ordinary sections; further (4) there are distinct lines of continuity clearly marked out by reticulum fibrils connecting the capillaries seen, *i. e.*, intercapillary channels potential and actual. It is the writer's concept that such channels serve normally as finer nutritive spaces and may, under stress of circumstances, open into spaces capable of carrying blood corpuscles. In other portions of the same slide, single red cells may be seen lying flattened in narrow clefts in the intercapillary reticulum lines. Fig. 16 shows two such clefts in an intertubular reticulum line, each of which contains red blood cells.

The same concept is also well illustrated in a study of the pancreas in Fig. 17. Here again continuity of reticulum fibrils and endothelial cells is seen; reticulum fibrils form the immediate lining of the capillary wall and, intercapillary reticulum-lined spaces are readily demonstrable. The identity of the reticulum fibrils and the basement membrane is again shown. The capillaries of the islets of Langerhans (not illustrated) also show a fibered cytoplasm and in favorable sections surface views may be seen. Here, focusing high at the surface of the capillary, very delicate slightly undulating black fibrils paralleling the longitudinal axis of the vessels are seen. At the lateral borders they are viewed several layers thick and show as darker lines.

The gastric mucosa (Fig. 18) illustrates the same principles outlined for the kidney and pancreas.

It is in connection with study of the heart muscle that the most striking support of the concept of finer capillary radicles is achieved. Fig. 19 shows an area of heart muscle in tangential section. Here a number of reticulum-lined capillaries with contained red blood cells are shown in cross-section and the reticulum-lined, intercommunicating spaces readily seen. Continuity of reticulum fibrils and the capillary lining cells is demonstrated. Selecting an edematous and congested heart muscle, Fig. 20, we see many of the potential spaces opened, in fact a veritable reticulum mesh containing red blood cells at various points. The photograph lacks sufficient detail to show these clearly. The same principles apply to the circulation in the adrenal and hypophysis, but inasmuch as a sinusoidal type of circu-

lation is generally conceded for these organs, they were omitted from detailed consideration. A glance at the section of thyroid will confirm the same concept for its circuit.

These preliminary studies indicate that the capillary bed is tremendously greater than generally conceived. The unopened bed is outlined by reticulum fibrils of endothelial cells. Such lines of communication probably ordinarily serve as channels for the conveyance of non-corpuscular elements of the blood, and, it is believed, may open under effective stimulus into channels of sufficient size to carry corpuscles. The reticulum fibers are in intimate contact with the parenchymatous cells of the organ and are, in fact, identified with the basement membrane in the kidney, pancreas, adrenal and gastric mucosa. Corner identified these fibers with the basement membrane in the kidney. It will be seen, then, that the capillary circuit is of an absolute character.

The concept is set forth as a morphological basis for the well known physiological capacity, as demonstrated by Krogh [21] and others, of the capillary bed to increase suddenly in extent.

The capillary bed is conceived as one of an absolute character, *i. e.*, one of the highest efficiency, touching the surface of all parenchymatous cells. A morphological background is given for the well known physiological capacity of the capillary bed alternately to open and rest and, under effective stimulus, to increase greatly in extent.

Summary and Conclusions

A new method of metallic impregnation is detailed which yields complete impregnation of mesenchymal, reticulum and collagen fibrils. An adequate polychrome counterstain may be superimposed upon the impregnated tissues. The mesenchymal cells possess a rich delicate fibrillar cytoplasm; the fibrils are readily impregnated by the method employed. Morphological support is given for the generally accepted concept that capillaries are formed *in situ* by a direct differentiation of the mesenchyme. This differentiation of capillaries in the mesenchyme is of a very simple character. The capillary endothelial cell remains in the embryo as a fiber-producing cell and this property and capacity persists into the mature organism. Both reticulin and collagen are fiber products derived from a common fibrillar mother substance and are undoubtedly chemically similar.

Reticulum fibers are demonstrated in capillaries in a wide variety of tissues, sufficiently wide to justify the concept that they are of universal occurrence in the capillary endothelium. Otherwise stated, reticulum may be identified as the fiber product of capillary endothelium. Similar fiber substance is present in the endothelial and reticulum cells of the lymph nodes. These cells, as the capillary endothelial cells, are little differentiated, direct descendants of the mesenchyme. Reticulum fibers are also present in the intersinusoidal or so-called reticulum cells of the splenic pulp and line the sinusoids of the liver. Reticulum fibers are a little changed descendant of the mesenchymal fibers.

Brief evidence is presented favoring the ability of reticulin to be transformed into collagen.

Reticulum is the most widespread and important supportive substance in the body. It is the scaffolding of cells and cell units. It serves the double purpose of microscopic cell support and the lining of capillary vascular channels.

By identifying reticulum with the capillary endothelium and obtaining sufficiently clear sections, the finer structure of the capillary bed is revealed. Reticulum fibers form the immediate lining of capillaries and minute reticulum-lined spaces are shown extending between and connecting the small capillaries as seen in ordinary sections. Such channels are considered to serve normally for the transfer of elements contained in the plasma of the blood and to be capable of enlarging or "opening up" under effective stimulus to a caliber sufficient to convey corpuscular elements. The endothelial reticulum is identified with the basement membrane in the kidney, pancreas, adrenal and gastric mucosa; this probably applies to basement membranes in general.

The author wishes to thank Dr. G. Y. Rush and Dr. C. L. Connor for stimulation and assistance received in the conduct of this study.

REFERENCES

1. Mall, F. P. Das Reticulirte Gewebe und Seine Beziehungen zu den Bindgewebsfibrillen. *Abhandl. d. Math.-Phys. Classe Königl. Sächs. Gesellch. d. Wissensch.*,1891, **17**, 229.
2. Corner, George W. On the widespread occurrence of reticular fibrils produced by capillary endothelium. *Contrib. Embryol.*, 1920, **9**, No. 272.
3. Mallory, F. B., and Parker, Frederick, Jr. Reticulum. *Am. J. Path.*, 1927, **3**, 515.
4. Kinney, Ethel May. A simple method for staining reticulum. *Arch. Path.*, 1928, **5**, 283.
5. McClung. Handbook of Microscopical Technique. Paul B. Hoeber, N. Y., 1929, 295.
6. Laidlaw, George F. Silver staining of the skin and of its tumors. *Am. J. Path.*, 1929, **5**, 239.
7. Downey, Hal. The structure and origin of the lymph sinuses of mammalian lymph nodes and their relations to endothelium and reticulum. *Hematologica*, 1922, **3**, 431.
8. Mall, Franklin B. On the development of the connective tissues from the connective-tissue syncytium. *Am. J. Anat.*, 1902, **1**, 329.
9. Hueck, W. Über das Mesenchym. *Beitr. z. path. Anat. u. z. allg. Pathol.*, 1920, **66**, 330.
10. Pulford, D. Schuyler, Jr. Neoplasms of the blood-lymph vascular system with special reference to endotheliomas. *Ann. Surg.*, 1925, **82**, 710.
11. McClure, C. F. W. The development of the lymphatic system in the light of the more recent investigations in the field of vasculogenesis. *Anat. Record*, 1915, **9**, 563.
12. McClure, C. F. W. The endothelium problem. *Anat. Record*, 1921, **22**, 219.
13. Reagan, F. P. Experimental studies on the origin of vascular endothelium and of erythrocytes. II. The local origin theory. *Am. J. Anat.*, 1917, **21**, 44.
14. Reagan, F. P. Vascularization phenomena in fragments of embryonic bodies completely isolated from yolksac blastoderm. *Anat. Record*, 1915, **9**, 329.
15. Stockard, C. R. An experimental study of the origin of blood and vascular endothelium in the teleost embryo. *Anat. Record*, 1915, **9**, 124.
16. Rössle, Robert, and Yoshida, Tanzo. Das Gitterfasergerüst der Lymphdrüsen unter normalen und pathologischen Verhältnissen. *Beitr. z. path. Anat. u. z. allg. Pathol.*, 1909, **45**, 110.
17. Russakoff, A. Über die Gitterfasern der Lunge unter normalen und pathologischen Verhältnissen. *Beitr. z. path. Anat. u. z. allg. Pathol.*, 1909, **45**, 476.

18. Miller, W. S. The reticulum of the lung. IV. Its presence in the reparative process of the tuberculous lesion of the lung with and without caseation. *Am. J. Path.*, 1927, **3**, 217.

19. Foot, Nathan Chandler. The endothelial phagocyte. A critical review. *Anat. Record*, 1925, **30**, 15.

20. Kupffer, C. Ueber Sternzellen der Leber. *Arch. f. mikr. Anat.*, 1876, **12**, 353.

21. Krogh, August. The Anatomy and Physiology of Capillaries. Yale University Press, 1929.

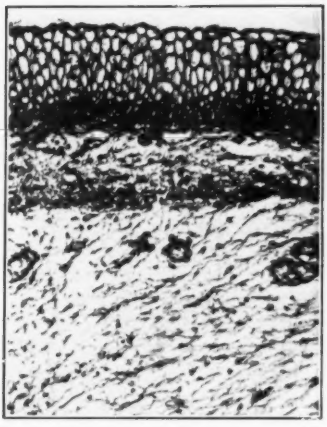
DESCRIPTION OF PLATES

PLATE 108

FIG. 1. Section of skin and subcutaneous tissue, 7.5 cm. pig embryo. Beneath the epidermis the mesenchyme is undergoing a transformation into fibrous tissue. The fibers have become rearranged longitudinally and now take the golden brown tone of collagen. The undifferentiated mesenchymal mesh is seen in the lower portion of the section. Stain: Method I. × 80.

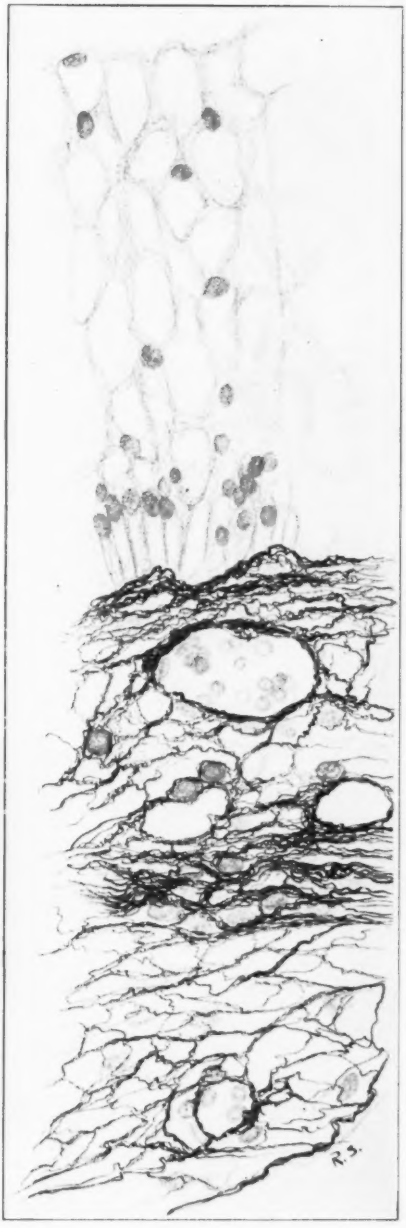
FIG. 2. Detail drawing taken from same slide as Fig. 1, showing the rearrangement of fibers in formation of the corium. The coarser longitudinal fibers take the rich golden brown tone of collagen. At the lower portion of the section is the looser fibrillar mesh of the mesenchyme. The wall of the small capillary in the mesenchyme is seen to be composed of cells, morphologically identical with those of the mesenchyme, the fibers of which not only form the wall of the capillary but anastomose freely with those of the surrounding mesenchyme. The fibers stain somewhat darker than those of the surrounding mesenchyme. If this capillary had "invaded" the mesenchyme it would have rearranged the fibers about it and certainly would not show delicate fibrils extending from its wall into the surrounding mesh.

FIG. 3. Section of the lung, 11 cm. pig embryo. Lymphatic spaces appear as simple clefts in the mesenchyme in the interlobular septae. Stain: Method I. (See Fig. 4 for detail.) × 80.



1

3



2

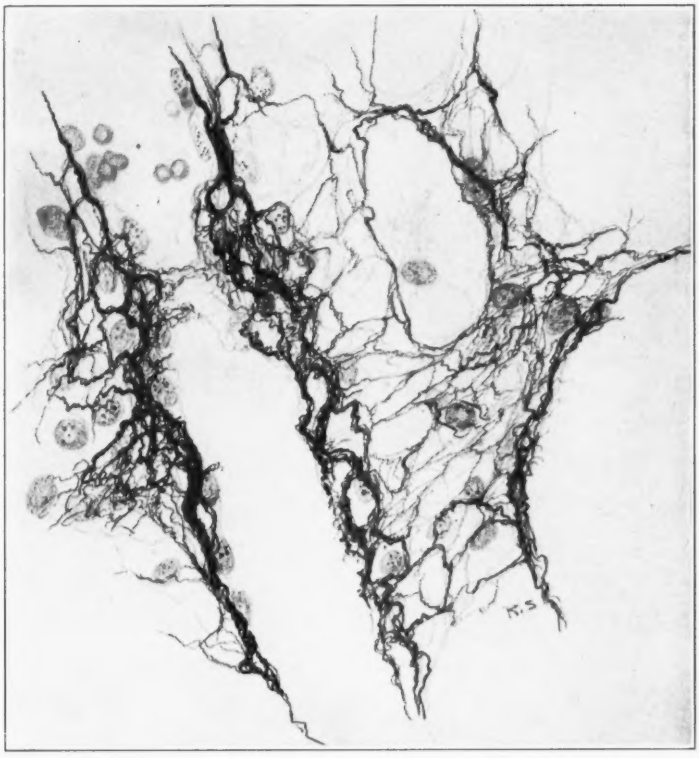
Rinehart Reticulum

PLATE 109

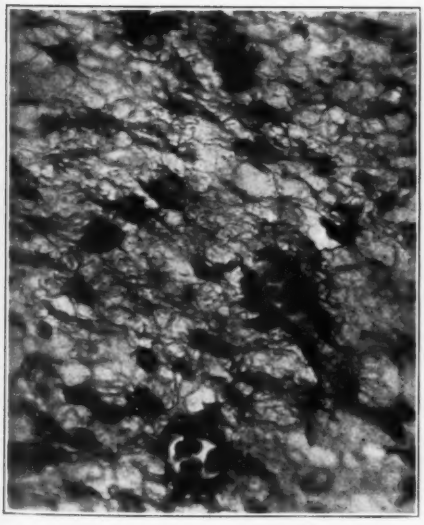
FIG. 4. Drawing from interlobular septum of 11 cm. pig embryo showing development of a small blood vessel in the mesenchyme. The anastomoses of the fibers forming the wall and of the intervening mesenchyme is clearly shown. The vessel is destined to be one of larger than capillary caliber. The arc at the right is a portion of the wall of a lymphatic cleft in the mesenchyme. Staln: Method I.

FIG. 5. Photomicrograph of two small capillaries in the corium in which the mesenchyme is being transformed into fibrous tissue. The fibers are assuming parallel arrangement and becoming coarser, and taking the deeper golden brown reaction of collagen. The fibers lining the two small capillaries are blacker than those of the surrounding mesenchyme. Stain: Method I. × 520.

FIG. 6. Group of tubercles in the liver from a case of miliary tuberculosis. The general architecture of the reticulum lining the sinusoids is shown. At the periphery of the tubercles the sinusoidal reticulum is seen to be coarsened. In the original sections the color contrast is striking; the coarsened reticulum takes the golden tone of collagen, the reticulum away from the tubercles stains black. The coarsening of the reticulum is well shown in the sinusoids intervening between the tubercle at the right of the photograph and the group in the center. Stain: Method I. × 80.



4



5

6

Rinehart

Reticulum

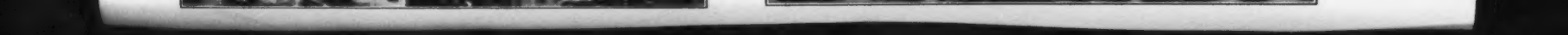
Plate 110

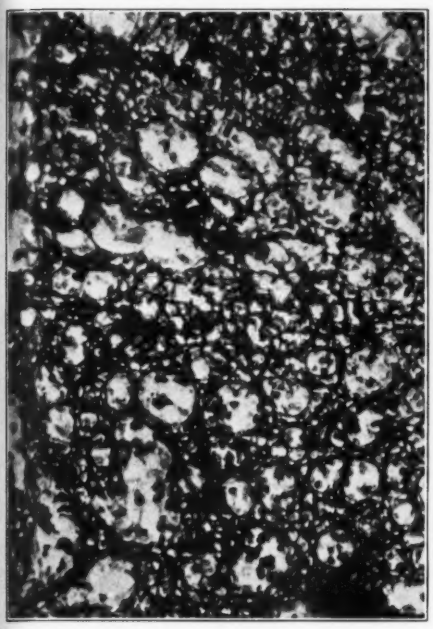
Fig. 7. Section of spleen in case of Banti's disease. A marked coarsening of the reticulum is seen. The coarse black areas shown in the photograph give the golden brown reaction of collagen. Those less coarse maintain the black reaction of reticulum. Stain: Method I. × 150.

Fig. 8. Section of essentially normal lymph node showing reticulum fibers continuous with the cells, lining and extending into the lymphoid sinus. Morphologically similar cells form the supportive stroma of the lymph cords. Other areas show reticulum fibers in the capillary endothelium. Stain: Method II. × 520.

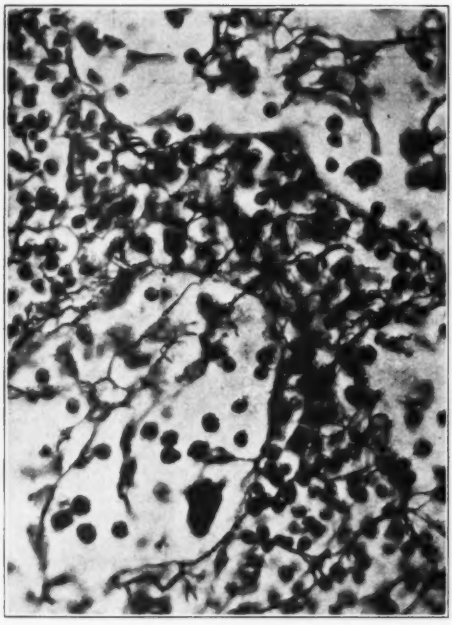
Fig. 9. Section of lung in an area of lesser reaction in a case of miliary tuberculosis. Reticulum fibers extending from cell nuclei and forming the immediate lining of two capillaries is well shown. One runs horizontally at the top of the photograph. The other in the upper right hand corner is cut transversely. Proliferating large mononuclear cells without fibers are seen attached to the capillary wall and free in the alveolar space. Stain: Method II. × 520.

Fig. 10. The drawing taken from the medulla of the adrenal clearly shows the continuity of reticulum fibers with the endothelial cells lining the capillaries and extending as the basement membrane of the medullary cells. This figure also illustrates the concept of the closed capillary bed described in detail in the kidney, pancreas and heart muscle. A triangular cleft outlined by reticulum containing a single red blood cell is seen near the center of the field. At the upper left angle this cleft is closed but the reticulum fibers continue as the basement membrane.

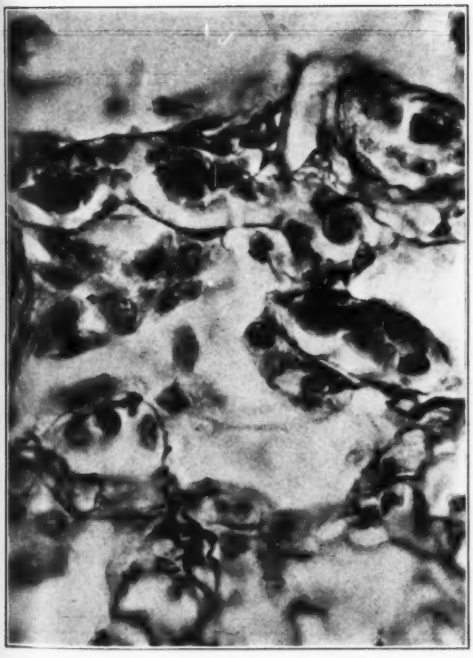




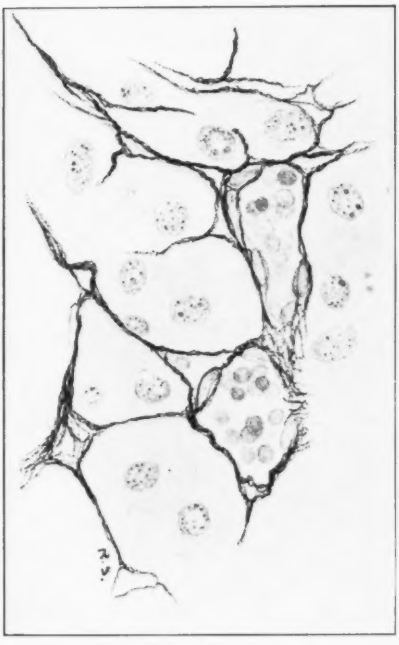
7



8



9



10

Rinehart

Reticulum

Plate III

Fig. 11. Photomicrograph of the adrenal cortex showing continuity of reticulum fibrils and endothelial cells lining the capillaries. Stain: Method I. × 780.

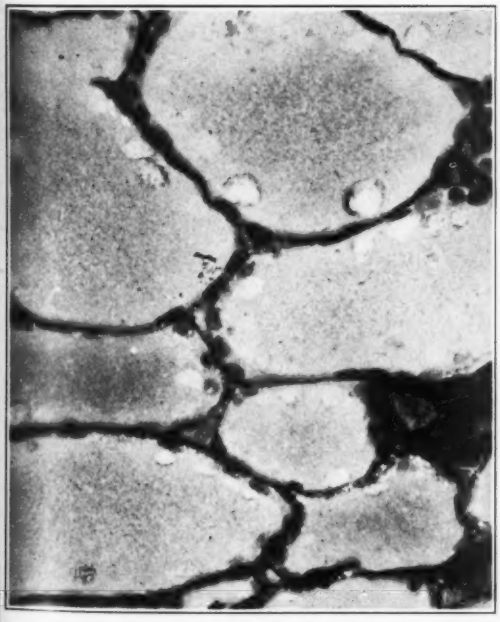
Fig. 12. Section of anterior lobe of the hypophysis showing the dense reticulum fibers lining the capillary spaces. Stain: Method I. × 520.

Fig. 13. Thyroid gland showing reticulum fibers lining open and closed capillary channels. A single cell layer existing between the vascular channel and the epithelium precludes the possibility of the reticulum being a fibroblastic layer. Reticulum fibers outline the open and potential capillary channels. Stain: Method I. × 520.

Fig. 14. Showing two small capillaries in the brain, one containing red cells, the other empty. Here in vessels, a single cell layer in thickness, are clearly shown reticulum fibrils forming the immediate vascular lining.

11

12



13

Rinehart

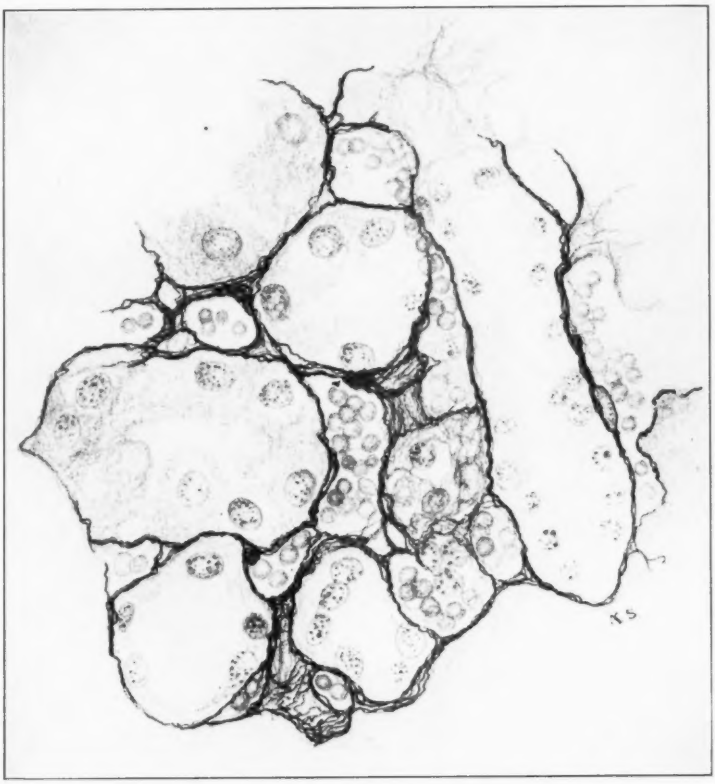
14

Reticulum

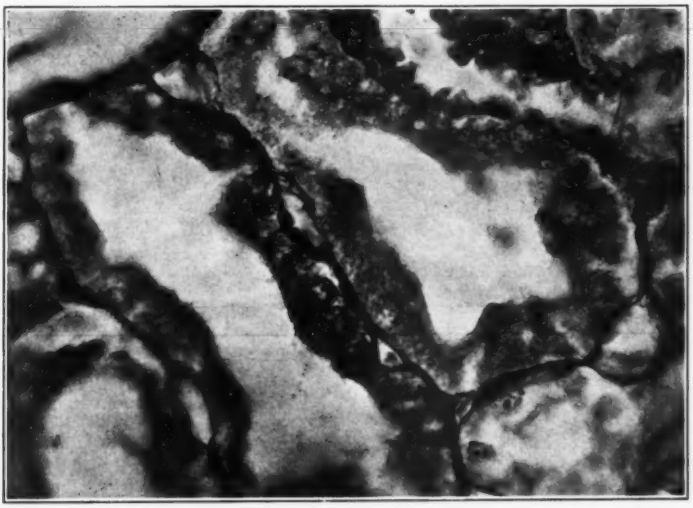
PLATE 112

FIG. 15. This drawing is taken from the kidney of a child. Here the following is shown: (1) continuity of delicate black fibers (reticulum fibers) with the endothelial cells in a perfectly simple one cell layer; (2) the identity of the basement membrane with the above described fibers; (3) oval, round and angular spaces, lined by reticulum fibers, containing red blood cells, capillaries as ordinarily seen, and, (4) connecting such spaces are fibers directly continuous with the capillary wall, forming lines of intercommunication between capillaries.

FIG. 16. Photomicrograph of the kidney illustrated in Fig. 20, showing two clefts in the reticulum "line" between two tubules. Red blood cells lie in each of these clefts. Stain: Method II. × 520.



15



16

Rinehart Reticulum

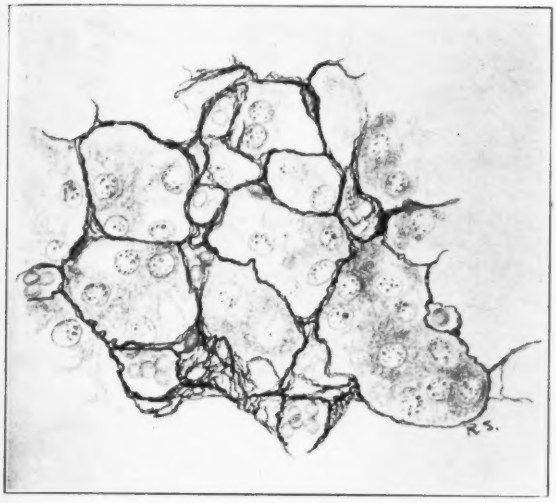
PLATE 113

FIG. 17. Drawing from pancreas of child, showing reticulum-lined capillary spaces containing red blood cells. The identity of the reticulum and the basement membrane is seen. Most of the capillary spaces in this field show only the reticulum lining, without the cells of origin. Endothelial nuclei with reticulum fibers in continuity are seen, lying between adjacent pancreatic acini, conceived as the lining of empty, collapsed capillary spaces. Potential but closed spaces are seen extending between demonstrable capillaries. Such fine channels are considered to serve for finer nutritive interchanges, and to be capable of carrying corpuscular elements of the blood under effective stimulus. Stain: Method II.

FIG. 18. Section of gastric mucosa, clearly showing reticulum fibers in the capillary endothelium and the identity of the latter with the basement membrane.

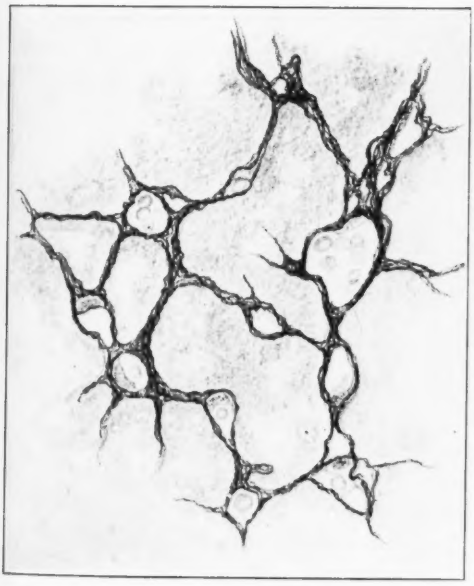
FIG. 19. Heart muscle in tangential section, showing continuity of reticulum fibrils with endothelial cells lining capillary spaces, and the continuation of these fibrils between capillaries forming minute passages for transfer of the blood plasma and conceived to be capable of "opening up" under effective stimulus for the conveyance of the corpuscular elements of the blood. Just above the center of the figure a single red blood cell is seen in a reticulum-lined endothelial cleft.

FIG. 20. Photomicrograph of a congested and edematous heart muscle, showing the reticulum-lined vascular spaces opened up. Although not showing clearly in the photograph, red blood cells are present at various points in the reticulum-lined capillary bed.

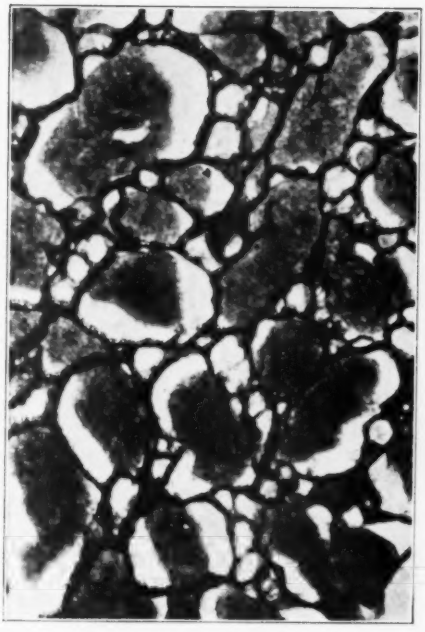


17

18



19



20

Rinehart Reticulum

DIAGNOSIS OF INTRACRANIAL TUMORS BY SUPRAVITAL TECHNIQUE *

LOUISE EISENHARDT, M. D. AND HARVEY CUSHING, M. D.
(From the Surgical Clinic, Peter Bent Brigham Hospital, Boston, Mass.)

With increasing satisfaction and confidence we have come to rely upon the supravital technique, devised by Sabin for the examination of the blood, as our most favored method for the making of pathological diagnoses of intracranial tumors. In this procedure the tissue is neither fixed nor frozen, but is simply prepared as a fresh smear of the living cells, and with experience immediate diagnoses can be secured which are often more dependable than those based on the study of stained tissue sections.

Our attention was first drawn to the possibilities of this method by Dr. Lawrence Kubie, who three years ago spent several weeks in the neurosurgical laboratory studying the cytological elements, more particularly the clasmatocytes, in the surgical specimens that happened to be supplied at that time. We have since then come to familiarize ourselves with the appearance of the tumors studied in this way and find that specimens, which have been prepared by the time-consuming methods in common use, fail in many instances to give the information we desire for purposes of classification. The method is of particular value in the differentiation of the gliomas into their various types, and believing that others will be interested in the subject, we propose to give a brief account of our experience with it.

Various methods for rapid microscopic diagnosis, particularly of malignant tumors elsewhere in the body, have been reported. Most of these, however, describe some process of fixation and the preparation of frozen sections. Hellwig [1] in 1926 advocated Wilson's method [2] of fixation in dextrin solution and staining of the sections with Unna's polychrome methylene blue, a procedure requiring about three minutes. They discovered that with the dextrin solution there was less shrinking and tearing of tissues than when formalin is used, and stated that individual cells were thereby more distinctly shown.

* Received for publication June 9, 1930.

Hoffheinz,[3] however, a year later expressed the opinion that the best results were obtained by the old method of quick fixation in hot formalin. He found that the dextrin solution caused sticky preparations which tended to form folds, and preferred the hematoxylin and eosin stain because it gave better color contrasts. Moreover, there was better preservation of the relationship of the various tissue structures. He concluded that the old method was a more dependable means of diagnosis, especially in obscure cases, justifying the five to ten minutes necessary for preparation.

Dudgeon and Patrick,[4] also in 1927, reported what they describe as a "wet-film method." The freshly-cut surface of the tissue is scraped and the juice thus obtained is spread on a slide and placed in Schaudinn's fluid for fixation. The films are then stained with haemalum and eosin. They allow eight to ten minutes for the complete process. In a series of 200 specimens they report successful results as compared with the control examination of sections prepared in paraffin by the usual technique.

MacCarty for years has used frozen sections of fresh unfixed tissue, stained with Terry's modification of Unna's polychrome methylene blue. In 1928 [5] he emphasized the importance of the cytological study of fresh unfixed material, and in a recent paper [6] states that the diagnosis of a malignant condition may be made from a single cell.

Taft and Ludlum [7] in a preliminary report have lately described a method for staining unfixed brain tissue with silver. The tissue is placed in argyrol, later washed in distilled water, and a film finally prepared.

In any method of quick diagnosis, the rapidity depends upon familiarity with a given procedure, the experience of the observer and the nature of the tissue. The morphological diagnosis of a tumor is occasionally very difficult whatever the method of preparation, and long and exhaustive study may be necessary before the final classification of the growth is possible.

Technique: The method, as stated, is that described in detail by Sabin [8] in her studies of living human blood cells. Its application to the more solid tissue of intracranial tumors is quite simple.

As soon as the tumor is exposed at operation, a fragment of it is routinely submitted for immediate examination. A minute portion is separated with dissecting instruments and placed on a glass slide.

The slide may be previously prepared with an even film of neutral red dye, or a mixture of neutral red and Janus green, the exact directions for which are given by Sabin. We have found that satisfactory results are obtained when a clean slide is used and a drop of aqueous solution of neutral red dye (1:10,000 or more) added directly to the tissue.

A cover glass is immediately placed over the tissue, which is carefully spread by gentle pressure on the cover glass, the rubber end of a pencil being convenient for the purpose. One learns by experience how much pressure to apply in making the smear. Some tumors are so soft that they spread almost like fluid, and anything more than the lightest touch damages the cells. Other tissues are very firm, fibrous or even gritty with calcium, requiring greater effort in flattening them out for a satisfactory film.

The preparation is sealed at once with a mixture of vaseline and paraffin around the edge of the cover glass, and is then examined microscopically in a warm box.

The actual preparation of the smear takes less than a minute.

There are one or two precautions which may be particularly commented upon at this point. If too strong a solution of the dye is used, the nuclei will be stained diffusely — a sign that the cells have been injured. The edges of the preparations are usually overstained in this manner. While overstaining does not necessarily so obscure a microscopic picture as to make a diagnosis uncertain, it must be avoided if one is interested in studying the cytological elements of these tumors.

Furthermore, the importance of making the preparations as soon as possible after the tissue has been removed should be emphasized whether or not an immediate microscopic report is imperative. A diagnosis may be made in most cases even though the smears are prepared an hour or several hours later, provided that the tissue has not been allowed to dry. However, there is no question but that the cells undergo changes on long standing, especially if the tumor is of a soft consistency. They become swollen and vacuolated, the cytoplasm markedly granulated, and the nuclei show degenerative changes. When extreme vacuolization occurs, one vacuole may fill the whole cell. In some areas cytoplasmic boundaries become unrecognizable, so that there is only disintegrated material to be seen.

As Kubie has pointed out in a study of the perivascular tissues of

the central nervous system by this technique,[9] preparations of solid tissue tend to be uneven. Some areas are too dense, and others too thin. But there are many fields in which the relations of the different structures are well preserved, and in the thinner portions individual cells which have become separated may be studied in minute detail.

In every case for comparison a portion of the same tissue is fixed to be subsequently sectioned and stained by the usual methods.

Advantages: Possibly the greatest value of the method herein presented lies in the opportunity it affords for the study of cells which have not undergone changes due to fixation or cutting, but which are seen under the microscope in their entirety while they are still living. The usual shrinkage and distortion of the tissues is thus avoided. Under high magnification (oil immersion) single cells, such as astrocytes and oligodendroglia, may be beautifully defined with all their delicate processes which are rarely to be seen intact in fixed histological sections. After fixation the cytoplasm is oftentimes destroyed, or in other instances it is demonstrated only by the use of special stains. An illustration of this is given in the photomicrographs of a recent oligodendroglioma (*cf.* Figs. 9 and 10) in which it may be seen how distinctly the cytoplasm of each cell is outlined in the fresh smear, while in the fixed preparations it has become changed beyond recognition.

Mitotic figures, too, may be identified in various phases in the supravital preparations of some of these tumors.

Not only are individual cells clearly shown, but, as has been pointed out, the relationship of the various structures in the tumor as a whole is well preserved, such as the formation of whorls and masses of cells in a meningioma, or the radiation of the cells of an ependymoma about a vessel and its branches, or the palisade arrangement of cells so characteristic of an acoustic neurinoma.

In invasive types of brain tumors myelin sheaths are often identified as curious irregular strands with bulbous enlargements and terminations sharply outlined by what appear as two closely parallel lines which are extremely refractive (*cf.* Fig. 6).

Collagen, too, has a distinctive appearance because of the refractive quality of its bands of wavy fibers.

Of particular interest are the histiocytes, which are frequently associated with such tumors as the rapidly growing gliomas, men-

ingiomas, and adenomas, and which are believed by Carrel [10] to influence growing cells by aiding in their nutrition. In these supravital preparations they may be observed during their activity to phagocytize the neutral red dye and store it in the digestive vacuoles within their cytoplasm. These vacuoles appear as round, highly refractive globules, which may increase greatly in size as they become filled with the inclusions of the dye. Clasmatocytes may be present in extraordinary numbers in a rapidly growing tumor like a medulloblastoma, showing evidence of great activity, or they may be scattered here and there, as in a pituitary adenoma, moving rather lazily among the tumor cells.

In some of the tumors there are other cells which contain highly refractive vacuoles in their cytoplasm, but which are non-motile and do not ingest the dye. These vacuoles are an evidence of cell degeneration. Such vacuolated cells are commonly found in acoustic neurinomas which are undergoing fatty degeneration, or occasionally in a meningioma they are conspicuous among masses of the tumor cells or within the whorls.

Diagnosis: In many cases the precise nature of the lesion is quite apparent to the operator from its gross appearance and location, as is usually true for example of such growths as the meningiomas. Under these circumstances the microscopic diagnosis is merely corroborative. On the other hand, there may be some uncertainty in the surgeon's mind as to whether, for example, an atypical soft vascular tumor exposed in the region of the sella turcica is a suprasellar meningioma or a pituitary adenoma, or whether a suspicious nodule apart from the main mass of tumor represents an implantation or metastasis, or whether a necrotic area consists possibly of degenerated brain in the neighborhood of the growth or actually is tumor.

Possibly the most important immediate differentiation that is needed is between the various types of gliomas, and although increased familiarity on the surgeon's part enables him to distinguish many of these tumors by their gross characteristics, this is only possible when he has become thoroughly familiar with its histological appearance. If the surgeon must wait for a week or two, or even for a few days before he can get a clear idea of the microscopic structure of the tumor he has seen at operation, his recollection of the gross appearance has necessarily become obscured. The immediate

knowledge of the cytological characteristics of a tumor is therefore of great value in teaching him to associate the histological picture with the gross appearance of the lesion. Furthermore it gives him an immediate indication of the prognosis so that he may modify his measures accordingly and determine what particular operative procedure to pursue and how radical or otherwise an extirpation of the lesion is desirable. Moreover if the growth is of a type benefited by roentgen-ray therapy, the latter may be instituted without delay.

This of course is less important with the tumors whose gross characteristics are better known, such as the acoustic neurinomas and the meningiomas, but even here operative difficulties of diagnosis may be encountered, a tumor, which was adherent to the dura proving histologically to be a carcinoma, whereas it was assumed to be a meningioma during the course of the operation.

Description of Tumors: A certain familiarity with the appearance of the different intracranial tumors in supravital preparations as contrasted with fixed preparations must be acquired before the various types of lesions can be distinguished with accuracy. A description of the microscopic appearance of the more common tumors when examined by supravital technique will therefore be given.

Gliomas: The gliomas represent over forty per cent of all intracranial tumors. The immediate differential diagnosis of the various types, as has been said, is important in indicating the prognosis and in determining the method of operative procedure. The three largest groups in the classified series are the astrocytomas, the glioblastomas, and the medulloblastomas, and they may accordingly be considered in the order of their frequency as follows.

Astrocytoma Fibrillare: When the tissue is quite tough and unyielding, and indeed it may contain calcium, the usual picture under the microscope is of a very dense network of neuroglial fibrillae which are rarely stained but appear as closely crisscrossing refractive processes, among which there are scattered nuclei. It is usually in the less dense areas along the margins of the preparation that the cytoplasm of the cells is most distinctly seen, and here one may find unmistakable astrocytes with their long processes extending in starlike fashion from the cell body, one of which not infrequently may be traced to a neighboring vessel.

Occasionally these tumors are soft in composition, the tissue spreading very easily. We have noticed especially in cases of such

fibrillary astrocytomas removed from the cerebellum in young children that one is likely to find instead of a dense feltwork of fibrillae an abundance of well preserved astrocytes throughout the preparation. The cells may be quite small with a few processes forming a delicate fibrillary meshwork. In Fig. 1 an astrocyte of this type is shown and particular attention may be called to the distinctness of the nucleus and chromatin particles, and the granules in the cytoplasm.

Astrocytoma Protoplasmaticum: The protoplasmic astrocytomas are usually soft. The most pronounced difference from the fibrillary type when examined microscopically is the general absence of neuroglial fibrillae. Furthermore they tend to undergo degenerative changes and become invasive, and it is not unusual to see among the fairly large oval nuclei of the tumor numerous degenerated cells packed with vacuoles, and clasmatocytes which have taken up the neutral red dye. Nerve fibers identified by their refractive myelin sheaths may also be found here and there. In this respect they are similar to the glioblastomas.

If the specimen is not too degenerated, however, the cytoplasm as well as the nucleus may be discerned, and in Fig. 2 the soft branching processes of a typical astrocyte may be seen extending out in various directions for a considerable distance. The nucleus appears darker in the photomicrograph than it should because of the attempt to reproduce satisfactorily the delicately outlined processes which were only slightly stained.

How much more informing the supravital preparation may be when contrasted with the Zenker-fixed specimen from the same tumor stained with phosphotungstic acid hematoxylin is evident from Figs. 3 and 4, which have been taken at a low magnification to show the general architecture rather than the cell type of the tumor.

Glioblastoma Multiforme: These rapidly growing tumors are composed of cells of various size and shape, including spongioblasts of all forms, astrocytes, round and spindle-shaped cells, which in a favorable piece of tissue are easily identified in the fresh smear. Multinucleated cells are present and mitotic figures may be demonstrated. However, many of these lesions are degenerated, and if the tissue examined is from a necrotic area such cells are not well shown, but instead the fields are occupied by great numbers of vacuolated cells which may be huge in size, filled with characteristic greenish

refractive globules which are often large. Active clasmatocytes ingesting the neutral red dye are also present (Fig. 5). Among them the nuclei of the tumor cells may be seen, and usually one finds scattered myelinated nerve fibers indicating the invasive nature of the growth.

It may be pointed out that occasionally a bit of tissue is submitted for diagnosis from the brain in the neighborhood of a tumor. Under these circumstances the myelinated nerve fibers (Fig. 6) present an unmistakable picture. At other times the tissue may come from the margin of the growth, and examination disclose neuroglial fibrillae and possibly astrocytes, which, however, represent a gliosis rather than the real tumor, so that a more representative piece of tissue is necessary in order not to confuse the diagnosis with that of a fibrillary astrocytoma.

Medulloblastoma: In Figs. 7 and 8 are shown for comparison the preparation by supravital technique and the preparation of the same tumor which had been fixed, cut and stained by the usual method, the nuclei being greatly shrunken and the cytoplasm practically indiscernible. These tumors are generally soft and should be spread gently. Examination by supravital technique shows a most distinctive picture of a rapidly growing exceedingly cellular tumor, composed of masses of small round cells with round or oval nuclei containing a fair amount of chromatin. The predominating type of cell found in the fresh preparations as seen under oil immersion (Fig. 7) is round rather than carrot-shaped. The cytoplasm is pale in contrast to the nucleus, which sometimes may be a little eccentric, but the cytoplasmic boundary is very well defined, as is evident in the photomicrograph. Mitoses in various phases are easily recognized and may be numerous with distinct chromosomes, as was true of this particular tumor from the roof of the fourth ventricle in a child. Occasionally spongioblasts and neuroblasts may be identified in these tumors, but in this case they were not present.

Hordes of clasmatocytes are often present in medulloblastomas and may be seen actively taking up the dye so that they stand out conspicuously among the tumor cells. We have already referred to Carrel's conclusions regarding their influence on the nutrition of growing cells, and in this particular tumor, which is obviously a very rapidly growing one, they were present in extraordinary numbers none happening to show in the photomicrograph.

Oligodendroglioma: Here again, in Figs. 9 and 10 the supravital preparation is shown in contrast with the fixed preparation of the same tumor stained by ordinary methods. These tumors are rare, representing only one per cent of the gliomas, so that only a few have been available for study by the supravital method of examination. When seen in the fresh smears they present a most interesting picture. Whereas in the fixed preparation (Fig. 10) the round though shrunken nuclei are seen, in no field of any section was the cytoplasm recognizable, though it may be noted that in some fixed preparations of similar lesions the nuclei may be surrounded by a halo. This may be contrasted with the fresh smear, which shows a typical field of the same growth at the same magnification (Fig. 9). The nuclei are spherical and unshrunken, containing quite abundant chromatin, and are surrounded by a pale cytoplasm which is very distinctly outlined. One may see in the photomicrograph how cellular the tumor is, but unlike the medulloblastomas, no mitoses are present. They are slowly growing tumors and almost invariably tend to undergo partial calcification.

The oligodendroglia are thought to develop from indifferent cells of ectodermal origin which may also differentiate into fibrillary astrocytomas. In a recent case of fibrillary astrocytoma of the cerebellum in a child of ten years, typical oligodendroglial cells were found in the supravital preparations of the tumor. In Fig. 11 two of these cells are shown with the slender processes characterized by swellings along their course and at their terminations. In fixed preparations the processes of these cells are not stained by usual methods, though they may be brought out by special silver stains as recently reported by Bailey and Bucy.[11]

Other Types of Gliomas: Of the remaining types of gliomas which form a smaller percentage of the classified group, one or two may be briefly referred to. There have been a few examples of ependymoma studied by this technique. They are highly cellular lesions and in the fresh smears the radiation of the cells around the vessels has been observed to particular advantage under low power, while under oil immersion extensions of the cells may be traced to the vessel walls. Blepharoplasten have not been distinguished in the tumors so far available for supravital study.

There have been two recent examples of spongioblastoma unipolare et bipolare. In Fig. 12 some of the individual cells are shown as they

appeared in the fresh preparation. They lie more or less parallel to one another, this arrangement having been quite consistent throughout all fields.

Pituitary Adenomas: The differential diagnosis between the chromophobe and chromophile types of pituitary adenomas is possible on examination of the supravital preparations. The chromophobe lesions are composed of masses or cords of epithelial cells which are round or polygonal in form with round or oval nuclei containing a variable amount of chromatin and usually well marked nucleoli (Fig. 13). The cytoplasm is very finely granular. Clasmatocytes are commonly found though they are generally not numerous and may be comparatively inactive. It may be noted that in the case of the youngest patient in the series with chromophobe adenoma, a girl who was first operated upon at the age of ten years, mitoses were observed in the fresh preparation of the tumor at a second operation five years later.

In the chromophile type the alpha granules which in fixed preparations are brought out best by special stains, such as Bailey's ethyl violet-orange G, are readily seen in the fresh smears as rather coarse granules most marked at the periphery of the cytoplasm. Multinucleated cells may be numerous and crescent-shaped cells are also found. There is considerable variation in the size of the cells. A rather striking picture was observed in a recent case of "fugitive acromegaly" upon examination of the fresh smear. Everywhere among other comparatively pale, delicately granular chromophobe cells there were great numbers of enormous cells which were especially conspicuous in appearance because of the very granular cytoplasm which stained deeply with the neutral red dye (Fig. 14). The nuclei were round with a prominent nucleolus, and some of the cells contained two nuclei.

Meningiomas: These tumors as a rule are unmistakable on examination by supravital technique. They vary in consistency from very soft to very firm lesions. One sees masses of cells with large nicely outlined oval, round or elongated nuclei which have a singularly typical appearance. Nucleoli are usually prominent. Whorl formation is well preserved in the fresh smears as may be seen in the photomicrograph which is taken at the same magnification as that of the fixed preparation of the same tumor (Figs. 15 and 16).

Even when calcium is present a smear satisfactory for diagnosis

may be obtained. The psammoma bodies under low power are conspicuous round refractive bodies, and when examined under oil immersion nuclei within some of these concentric concretions may be identified.

Occasionally there is evidence of degeneration, and in one of our recent cases the cytoplasm of the tumor cells within and outside of the whorls was filled with very fine greenish globules.

Clasmatocytes are associated with some of the meningiomas, as has been pointed out, and they may be observed ingesting the neutral red dye.

Although in most cases the gross appearance of a meningioma leaves no doubt in the surgeon's mind as to its histological nature, in rare instances the gross features may be misleading. On a recent occasion, for example, a tumor which was adherent to the dura and was regarded as a meningioma proved by supravital preparation to be a carcinoma.

Acoustic Neurinomas: The acoustic neurinomas are usually firm in composition. If the tissue is well preserved and cellular, the fresh smear shows oval or elongated nuclei running along in fibrillary strands in the palisade and occasionally whorl-like arrangement which is typical of these growths. The cytoplasm of the cells in such fields is ordinarily not well defined, but the general architecture of the tumor is unmistakable.

Frequently the tissue submitted has a yellowish fatty appearance, and when examined by supravital technique it shows the characteristic arrangement mentioned above, but the cytoplasm is more distinct because it is packed with fine greenish refractive vacuoles. The cells are commonly elongated and spindle-shaped, with fibrillary extensions at their extremities. When the tumor is extremely degenerated, however, many of the cells show only the nuclei, with globules of fat trailing along the fibrillary material present.

Conclusions

The supravital technique has been adopted as the most favored routine method of diagnosing and classifying tumors of the central nervous system, it being of particular value in the cytological differentiation of the various types of gliomata.

Not only can an immediate diagnosis be given to the surgeon so that he may learn to associate the microscopic type of the lesion

with its gross appearance at the operating table, but a permanent photographic record of the fresh preparations can be made for comparison with the permanent section of the fixed tissue.

The supravital method makes it possible for the examiner to see the cells with their cytoplasm and processes intact and gives pictures which are wholly unfamiliar to those who have only studied these cells in fixed sections.

REFERENCES

1. Hellwig, C. A. Zur histologischen Kontrolle der Operation. *Zentralbl. f. Chir.*, 1926, **53**, 2647–2649.
2. Wilson, L. B. A method for the rapid preparation of fresh tissues for the microscope. *J. A. M. A.*, 1905, **45**, 1737.
3. Hoffheinz, S. Zur Technik der histologischen Schnelldiagnose. *Zentralbl. f. Chir.*, 1927, **54**, 2498–2501.
4. Dudgeon, L. S., and Patrick, C. V. A new method for the rapid microscopical diagnosis of tumours: with an account of 200 cases so examined. *Brit. J. Surg.*, 1927, **15**, 250–261.
5. MacCarty, W. C. A cytologic key to the diagnosis and prognosis of neoplasms. *J. Lab. & Clin. Med.*, 1928, **13**, 354–365.
6. MacCarty, W. C. The diagnostic reliability of frozen sections. *Am. J. Path.*, 1929, **5**, 377–380.
7. Taft, A. E., and Ludlum, S. DeW. A method for staining unfixed brain tissue with silver. *Proc. Soc. Exper. Biol. & Med.*, 1930, **27**, 582–585.
8. Sabin, F. R. Studies of living human blood-cells. *Bull. Johns Hopkins Hosp.*, 1923, **34**, 277–288.
9. Kubie, L. S. A study of the perivascular tissues of the central nervous system, with the supravital technique. *J. Exper. Med.*, 1927, **46**, 615–626.
10. Carrel, A., and Ebeling, A. H. The fundamental properties of the fibroblast and the macrophage; malignant fibroblast of "sarcoma 10" of the Crocker Foundation. *J. Exper. Med.*, 1928, **48**, 105–123.
11. Bailey, P., and Bucy, P. C. Oligodendrogliomas of the brain. *J. Path. & Bact.*, 1929, **32**, 735–751.

DESCRIPTION OF PLATES

PLATE 114

FIG. 1. Supravital preparation showing a single fibrillary astrocyte. Note the distinctness of the granules in the cytoplasm. × 850.

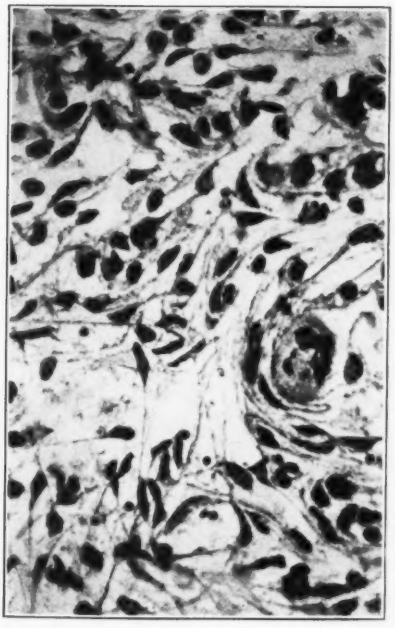
FIG. 2. Supravital preparation showing a single protoplasmic astrocyte. Note the numerous soft branching processes. × 850.

FIG. 3. Supravital preparation of an astrocytoma. For comparison with Fig. 4. × 300.

FIG. 4. Zenker-fixed preparation of same tumor as in Fig. 3 (phosphotungstic acid hematoxylin stain). × 300.

1

2



3

4

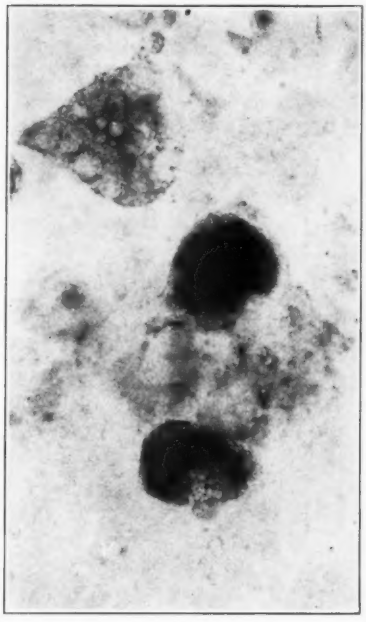
PLATE 115

FIG. 5. Supravital preparation showing vacuolated cells in a rapidly growing glioma. The upper cell is inactive, while two histiocytes below have ingested particles of neutral red dye. × 600.

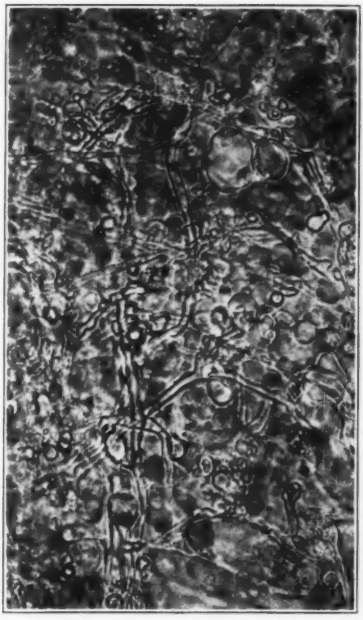
FIG. 6. Supravita. preparation of fragment of brain to illustrate the typical appearance of myelinated nerve fibers. They are highly refractive and are often found among the cells of an invasive tumor. × 600.

FIG. 7. Supravital preparation of a medulloblastoma showing clearly defined cytoplasm (arrows) and uniformly round shape of cells. Two mitotic figures are included in the field. × 850.

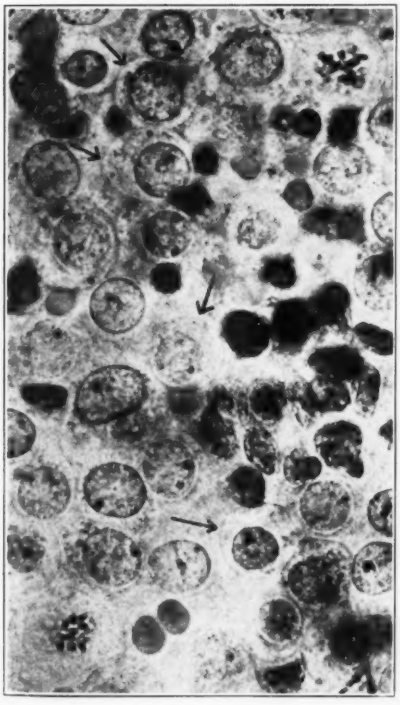
FIG. 8. Formalin-fixed preparation of same tumor as in Fig. 7 (hematoxylin-eosin stain). The cytoplasm has been almost wholly lost and the nuclei are greatly shrunken. × 850.



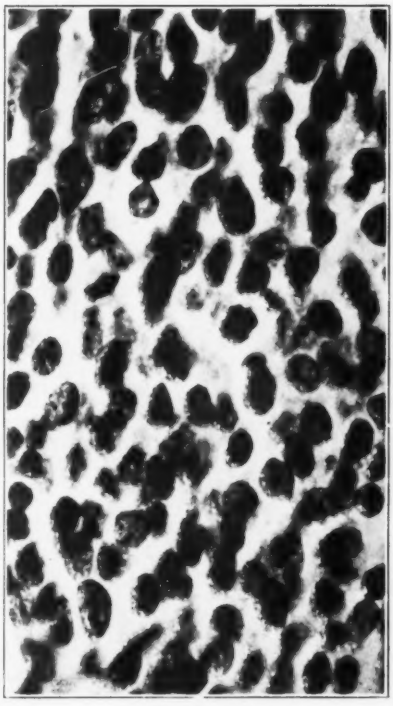
5



6



7



8

Eisenhardt and Cushing Diagnosis of Intracranial Tumors

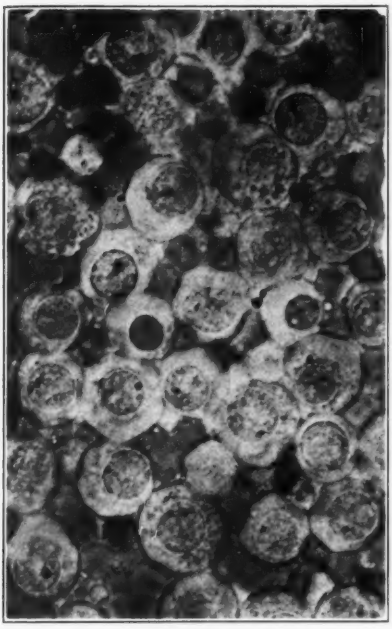
Plate 116

Fig. 9. Supravital preparation of an oligodendroglioma to show the sharply outlined cytoplasm of the cells and their spherical nuclei. For comparison with Fig. 10. × 850.

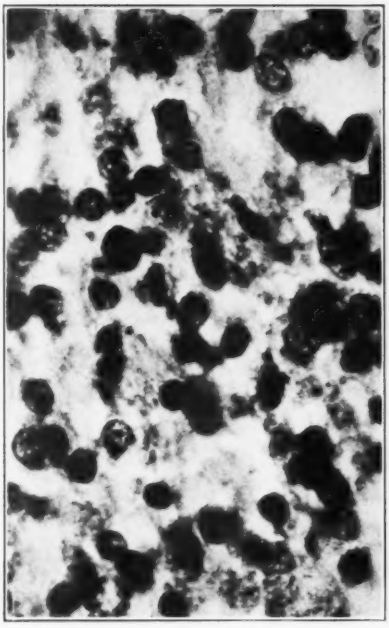
Fig. 10. Zenker-fixed preparation of same tumor as in Fig. 9 (eosin-methylene blue). The cytoplasm was not recognizable in any field of several sections studied. The nuclei are much shrunken. × 850.

Fig. 11. Supravital preparation showing two isolated oligodendroglial cells from a midline cerebellar fibrillary astrocytoma, with typical swellings along their processes. × 850.

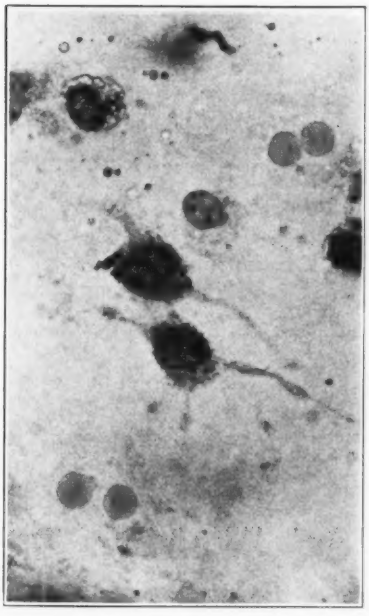
Fig. 12. Supravital preparation of a spongioblastoma unipolare et bipolare, showing several of the individual cells in characteristic parallel arrangement. × 850.



9



10



11

12

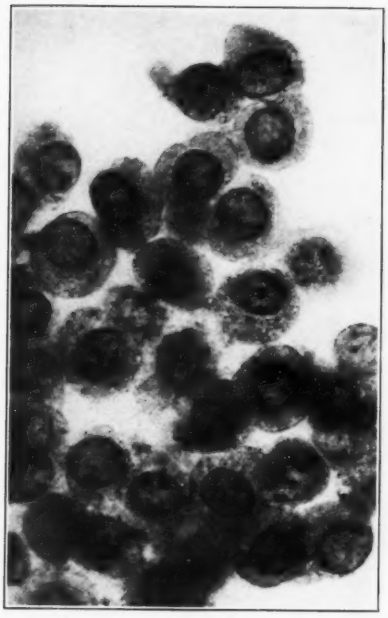
PLATE 117

FIG. 13. Supravital preparation of a chromophobe pituitary adenoma showing a group of cells of about the same size with very finely granular cytoplasm. × 850.

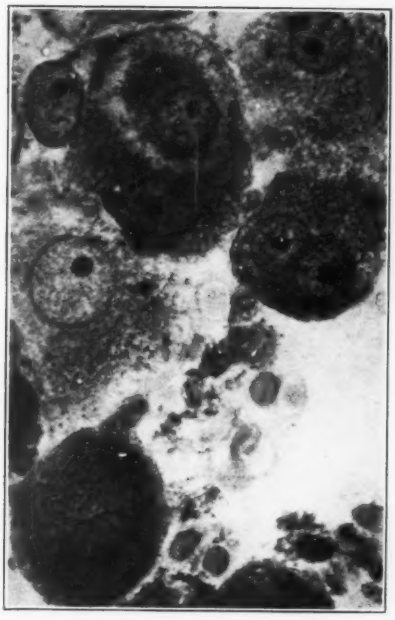
FIG. 14. Supravital preparation of a pituitary adenoma of mixed type. Note the size of the cells as compared with those in Fig. 13, and the granular cytoplasm stained by neutral red. One cell with two nuclei is shown. × 850.

FIG. 15. Supravital preparation of a meningioma to show how well whorl formation is preserved. × 600.

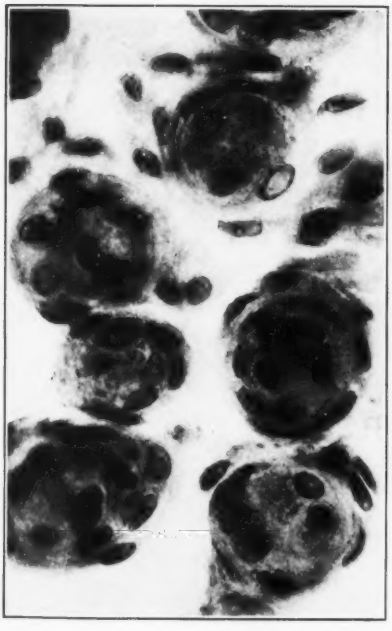
FIG. 16. Zenker-fixed preparation of same tumor as in Fig. 15 (eosin-methylene blue stain). × 600.



13



14



15

16

SMALL CELL CARCINOMAS OF THE LUNG*

HOWARD T. KARSNER, M.D., AND OTTO SAPHIR, M.D.

(From the Institute of Pathology, Western Reserve University, Cleveland, Ohio)

With the increasing number of reports upon primary carcinoma of the lung, views as to classification have changed. Classification upon the basis of gross morbid anatomy has been largely abandoned because of wide variations in form, which overlap the classes, apparently as the result of local extensions and regional metastases. Classification as to point of origin is unsatisfactory because there have been no convincing demonstrations of origin from any part of the lung other than bronchi and bronchioles.

Classification upon the basis of the histological character is not without its difficulties, but is more satisfactory than the other two modes. Squamous epitheliomas and adenocarcinomas can be readily identified. Even when the epitheliomas show no keratinization the other features are usually sufficient for a diagnosis. Often the adenocarcinomas show only a few areas of acinus formation, which may not be found in only a single section. Of the undifferentiated cancers some are made up of polygonal, cuboidal or cylindrical cells and can only be called carcinoma simplex. Others show a curious tendency to the formation of bizarre multinucleated giant cells, but it is doubtful that these justify a separate classification because such cells are found in undifferentiated and differentiated tumors of our series. Most common among the undifferentiated forms are those cancers which have been called transitional cell, oat-cell and small cell carcinomas.

Practically without exception this last group of tumors is made up of small spindle cells and small round cells, one or the other predominating in different parts of the tumor and its metastases. In the spindle cell areas a certain degree of nuclear palisading is noted but not with the same regularity of arrangement commonly seen in the neurofibroma (Schwannoma). The cells are likely to be grouped in large nests, bounded by irregular, well vascularized septa of mature

* Received for publication June 5, 1930.

connective tissue, and the tumor is thus said to be alveolated. In the midst of the cell masses there may be a papilliform strand of connective tissue fibers with one or two thin-walled blood vessels and the tumor cells appear to grow in parallel bundles from this fibrous tissue. Nuclear character varies greatly. The spindle cells usually have vesicular nuclei and the round cells usually densely chromatic nuclei, but the reverse may be true. Mitotic figures are not commonly abundant.

Ewing's [1] article on lymphoepithelioma describes tumors of the nasopharynx which histologically resemble transitional cell carcinoma, but the structure of bronchi and bronchioles gives no ground for assuming that the small cell cancers of the lung belong in the group of lymphoepithelioma.

Only within recent years have the small cell tumors of the lung been accepted as cancers, although Turnbull, who has seen an extraordinary number of lung tumors at The London Hospital, is quoted by Simpson [2] as having been of this opinion for many years. That they are not sarcomas has been concluded because of cellular arrangement, vascularization, connective tissue relations, gross characters resembling obvious carcinomas and distribution of metastases.

The subject is of interest in connection with the Schneeberg lung cancers. Clinically the disease was described in 1770 (see Uhlig[3]). In 1879 Härting and Hesse described cases which came to autopsy. Schmorl [4] states that these were considered to be lymphosarcoma by Wagner, by Weigert and by Anke. Arnstein [5] pointed out clearly that the small cell metastases of his case were epithelial. Schmorl quoted Uhlig as having demonstrated that one of the Härting-Hesse cases was a small cell carcinoma and the other was combined with an "Endothelkrebs." With this background the impression has apparently been gained that the Schneeberg tumors are commonly of the small cell type. Schmorl's examination of twenty-one Schneeberg lung cancers showed that only three of them were small cell cancers.

In some clinics the small cell tumors constitute the majority of the lung cancers, but in others their number is exceeded by the squamous epitheliomas. Of twenty-five cases of primary carcinoma of the lung observed by us at City and Lakeside Hospitals, demonstrated by complete autopsy, thirteen were squamous epitheliomas,

six were small cell carcinomas, four were adenocarcinoma and two were classified as carcinoma simplex.

It is at least possible that the study of primary carcinoma of the lung may be advanced by detailed examination of the different forms encountered. For this purpose the cases of small cell carcinoma have been selected. The information provided by Weller [6] and by Simpson provides us with data for larger comparison.

Primary cancer of the lung occurs between 20 and 80 plus years, with the greatest incidence between 50 and 60 years. In our twenty-five cases the ages were between 38 and 76 years. The small cell cancers occurred between 40 and 72 years, distributed at 40, 41, 41, 58, 64, 72 years. There is then no striking change of age incidence.

Although statistics differ, in general it may be said that the sex ratio of primary cancer of the lung is four males to one female. In our cases of small cell carcinoma four were in males and two in females.

Of our twenty-five cases nineteen were in whites and six were in negroes. Of the small cell cancers, five were in whites and one in a negro.

Duration of symptoms is based upon much human uncertainty. In Simpson's 139 cases, the average duration was seven months. Only seven lived more than two years and two more than four years. In our twenty-five cases, the duration was ten days to two and a third years. In the small cell cancer cases it was three weeks to two years.

Generally it is found that the right lung is involved slightly more often than the left. In one of our six cases of small cell carcinoma, the lesion was discovered microscopically and the side not identified. Of the others, three were on the right side and two on the left.

A study of occupation and symptoms shows nothing in which the small cell cancers differ materially from the others. Moderate anemia, leucocytosis, fever, cough, weakness, anorexia, dyspnea, hemoptysis and pain in the chest were present about equally in both groups.

The correct clinical diagnosis was made in ten of the twenty-five cases, but in the cases of small cell cancers in only one of six instances. In one of these cases it was not to be expected that the diagnosis should be made because the tumor was only about 5 mm. in diameter.

Grossly, with the possible exception of the tumor discovered microscopically, all the small cell cancers were situated at the hilum. Two showed multiple small nodules throughout the lung. The other three were masses 6 to 12 cm. in diameter near the hilum. One case showed extensive bronchiectasis and one showed an area of necrosis (not gangrenous) 6 to 8 cm. in diameter, distal to the tumor. Generally, the tumors were of gray color, clearly invasive, firm, cut with resistance and showed a firm, pale gray or yellowish gray, moist cut surface. Vascularization was not marked and hemorrhage not noteworthy. Necrosis occurred in the larger masses as it did in carcinoma in general.

Extrathoracic metastases were proportionately more frequent in our small series of small cell cancers than in the others. Especially notable is the fact that whereas four of the nineteen other cancers showed extensive mediastinal metastasis, three of the six small cell cancers showed marked involvement of that region. Two of these were diagnosed clinically as mediastinal sarcoma. The situation in our cases, as is true of such tumors generally, was especially in the upper posterior mediastinum with anterior displacement of the trachea. This type of metastasis has been emphasized by others and Barnard [7] goes so far as to state that "the so-called 'oat-celled sarcoma' of the mediastinum is a medullary carcinoma of bronchi." Shennan [8] describes thirteen cases of small cell cancer of the lung, one of which (No. 8) is not entirely acceptable to us as of this category. All of these showed some degree of mediastinal involvement and in seven of them it was marked. Duguid and Kennedy [9] conclude "that oat-cell forms in a mediastinal tumor must not always be interpreted as indications of bronchial origin," and report one case originating in a thymic tumor and one originating in the mediastinal lymph nodes. Schuster,[10] without publishing the figures, states that "there is no relation between the type of growth and the size of the mediastinal mass." Maxwell [11] in a series of 239 cases of primary malignant tumor in the thorax, of which 135 were examined microscopically, found forty-seven cases of "obvious" carcinoma and sixty-four cases of oat-cell tumor. Mediastinal involvement was found in about 60 per cent of the small cell tumors as compared with about 55 per cent of the other cancers. Huguenin [12] speaks of "tumeurs médiastino-pulmonaires qui se présentent fréquemment sous l'aspect de tumeurs à petites cellules." It is probable

therefore that mediastinal involvement is somewhat more frequent in connection with the small cell cancers than is true of other cancers of the lung.

Discussion of the origin of primary cancers of the lung has been of particular importance as concerns the small cell tumors, for this is the type which Huguenin thought to be derived from alveolar lining. Weller, in his review, states that "proof of the origin of carcinoma of the lung from histologically unaltered alveolar epithelium is lacking." Subsequently Schuster concluded that "the existence of alveolar carcinoma is not proven." Maxwell, referring to the sixty-four small cell carcinomas he studied, states that "it has not been shown that any of the tumors in this series arose directly in the epithelial lining of the pulmonary alveoli." Huguenin, Foulon and Delarue,[13] as well as Huguenin, appear to admit some doubt as to Huguenin's original contention. All writers on this subject hope for what Weller calls the "fortuitous discovery of early examples in the course of routine autopsies," or the "hazard heureux de coupe" of Huguenin. Certainly the modern literature shows agreement with Weller that cell type of the tumor is not a criterion of point of origin.

Figs. 1, 2, 3 and 4 are from a small cell cancer found by Dr. Alan Moritz in the routine sections of the lung in a case of chronic diffuse bronchiectasis. In addition to masses of oval cells in the lung tissue a large space shows a lining of multiple layers of cells of the same type. That this space is not alveolar is indicated by the fact that its walls contain much smooth muscle, as shown by the round ended cylindrical nuclei. The Van Gieson technique shows many yellow-stained fibrils in the wall and the Verhoeff elastica stain several layers of elastic fibrils in irregular arrangement. The Mallory connective tissue stain shows spaces between the connective tissue fibrils. It must be a bronchiole. A slightly larger bronchiole shows squamous metaplasia without keratinization. It is probable, but not proved, that the multiple layers of oval cells represent a diffuse proliferation, but this does not exclude the possibility of extension of the cancer over the bronchiolar surface. No other focus of origin could be found in many sections, nor could a similar multiplication of layers be found in any of the clearly distinguishable alveoli. The Bielschowsky-Foot stain [14] (Fig. 2) shows no reticulum in the surface cells or in the deep cancer nodules. The origin of this lesion appears to be in a bronchiole and not in a bronchus with cartilaginous

walls. Huguenin has suggested that origin from a bronchiole might easily be confused subsequently with origin from alveolar lining cells.

The problem of multicentric or unicentric origin is not solved by this observation. The bronchiolar surface involved is about 1 mm. in length. To this extent the origin appears to be somewhat diffuse as compared to the possibility of origin in a few cells. If multicentric in this instance, the number of centra must be small. Broadly speaking, this tumor can be regarded as unicentric. The examination of our entire series of lung carcinomas indicates that they originate in a bronchus or bronchiole and that multiplicity of tumors in the same lung is the result of metastasis rather than multicentric origin. This view is supported by the early invasion of lymphatics, frequent invasion of veins and occasional invasion of arteries, common to lung cancers.

The gross morbid anatomy of small cell carcinomas of the lung differs from that of other carcinomas of the lung in only two particulars. The small cell cancers are generally somewhat firmer and show a slightly greater disposition to produce large mediastinal masses than the other cancers.

Microscopically, in addition to cell type, the vascularization and desmoplastic activity of the small cell cancers is striking. The blood vessels are numerous in the pulmonary and metastatic tumors. They have thin walls, with little or no musculature and appear in delicate or heavy bands of connective tissue throughout the tumor masses. The bare capillaries or vascular slits of sarcoma are not found. With the Van Gieson stain the amount of fibrous connective tissue is seen to be in excess of what might be expected from viewing the same tissue stained with hematoxylin and eosin. Not only are there heavy bands which give the tumor an alveolated appearance, but within the tumor cell masses are many more or less delicate short or long bundles. As compared with other cancers of the lung, the amount of connective tissue in the small cell cancers appears to be greater and its distribution more diffuse.

Three of our small cell cancers have been stained with the Foot modification and one with the Maresch modification of the Bielschowsky technique. Whereas the cell masses show fibrils of connective tissue, they are devoid of reticulum. This is entirely independent of rapidity of growth as judged by clinical history or gen-

eral appearance of the tumor. In no instance were reticulum fibrils found except as occasional strands connected with the reticulum of the surrounding host tissue. There is no indication whatever that the tumor cells have any capacity to form reticulum. This demonstration is in accord with that made as early as 1913 by Arnstein. Although rapidly growing sarcomas may be poor in reticulum, the lymphatic group, with which the small cell cancers have been confused, usually show a striking tendency in this direction, in sharp contrast to the small cell cancers.

The cells of the tumor appear in masses which resemble pure cultures of the cells (Huguenin). This appearance is conspicuous in hematoxylin-eosin and Bielschowsky preparations, but in Van Gieson preparations the illusion is destroyed by the presence of the connective tissue fibrils. Parallel rows of oval or spindle cells are attached to the denser connective tissue bands. These are continuous with masses of round cells or of spindle or oval cells which often occur in bundles with a certain suggestion of palisading of nuclei. There is no reason for suspecting that the round cells are simply cross-sections of spindle cells because they occur in large masses without spindle cells in association. The fact that areas of tumor necrosis are usually surrounded by round cells and that densely chromatic small nuclei, probably pyknotic, are frequent in the round cells suggests that some of these cells are the seat of regressive pathological change. Thus, they may be regressive forms of other round cells or of spindle cells. Variability of nuclear staining is a notable feature of both spindle and round cells. Nuclei may appear to be vesicular or densely chromatic. Since the nuclei of bronchiolar epithelium and the connective tissues stain normally in the sections it is probable that the tumor cell nuclei vary in chromatin content as indicated by the staining. In a specimen removed by the bronchoscope, the same irregularity of nuclear character was observed. No autopsy was permitted in this case and although the diagnosis was practically certain the case is not included in our series. Admitting it as a small cell cancer of the lung excludes the irregularity of nuclear staining as a postmortem change. The nuclei of the round cells are of the order of twice the diameter of an erythrocyte. Those of the spindle cells are of the order of one erythrocyte in width and two erythrocytes in length. Multiple nuclei are infrequent. The few mitotic figures observed are not abnormal.

Although others have observed a considerable differentiation of the cells, only one of our cases showed in one field cells which could be regarded as cuboidal or low cylindrical. Generally speaking, these small cell tumors show little or no further differentiation.

Conclusions

1. Small cell primary tumors of the lungs or bronchi are epithelial in character, as indicated by cell arrangement, relation of connective tissues and blood vessels, and complete absence of capacity to form reticulum.

2. Small cell cancers of the lung originate in bronchi or bronchioles and are probably unicentric in origin.

3. Small cell cancers of the lung more frequently produce large mediastinal masses than do other cancers of this organ and are firmer in consistency, but in other clinical and gross pathological aspects show no distinctive characters.

REFERENCES

1. Ewing, J. Lymphoepithelioma. *Am. J. Path.*, 1929, **5**, 99.
2. Simpson, S. L. Primary carcinoma of the lung. *Quart. J. Med.*, 1929, **22**, 413.
3. Uhlig, M. Ueber den Schneeberg Lungenkrebs. *Virchows Arch. f. path. Anat.*, 1921, **230**, 76.
4. Schmorl, G. (See Rostoki and Saupe.) Die Bergkrankheit der Erzbergleute in Schneeberg in Sachsen ("Schneeberger Lungenkrebs"). *Ztschr. f. Krebsforsch.*, 1926, **23**, 360.

   Thiele, Rostoki, Saupe and Schmorl. Ueber den Schneeberger Lungenkrebs, *München med. Wchnschr.*, 1924, **71**, 24.
5. Arnstein, A. Ueber den sogenannten "Schneeberger Lungenkrebs." *Verhandl. d. deutsch. path. Gesellsch.*, 1913, **16**, 332; *Wien. klin. Wchnschr.*, 1913, **26**, 752.
6. Weller, C. V. The pathology of primary carcinoma of the lung. *Arch. Path.*, 1929, **7**, 478; Entdifferentiation in primary carcinoma of the bronchi and lungs. *J. Cancer Research*, 1929, **13**, 218; The pathology of certain signs and symptoms in primary carcinoma of the lung: illustrative cases. *Ann. Int. Med.*, 1929, **2**, 725.
7. Barnard, W. G. The nature of the "oat-celled sarcoma" of the mediastinum. *J. Path. & Bact.*, 1926, **29**, 241.
8. Shennan, T. Tumours of mediastinum and lung. *J. Path. & Bact.*, 1928, **31**, 365.
9. Duguid, J. B., and Kennedy, A. M. Oat-cell tumours of mediastinal glands. *J. Path. & Bact.*, 1930, **33**, 93.
10. Schuster, N. H. Aetiology and pathology of primary lung tumours. *J. Path. & Bact.*, 1929, **32**, 799.
11. Maxwell, J. Primary malignant intrathoracic tumours. *J. Path. & Bact.*, 1930, **33**, 233.
12. Huguenin, R. Les aspects histologiques des cancers primitifs du poumon. *Ann. d'Anat. Path. et d'Anat. Norm.*, 1929, **6**, 241.
13. Huguenin, R., Foulon, P., and Delarue, J. Le revêtement de l'alvéole pulmonaire ses destinées pathologiques. *Ann. d'Anat. Path. et d'Anat. Norm.*, 1929, **6**, 775.
14. Foot, N. C. Technic for demonstrating reticulum fibers in Zenker-fixed paraffin sections. *J. Lab. & Clin. Med.*, 1924, **9**, 777. See also McClung, C. E., Handbook of Microscopical Technique, New York, 1929, 294.
15. Foot, N. C., and Day, H. A. The occurrence of reticulum in tumors. *Am. J. Path.*, 1925, **1**, 431.

DESCRIPTION OF PLATES

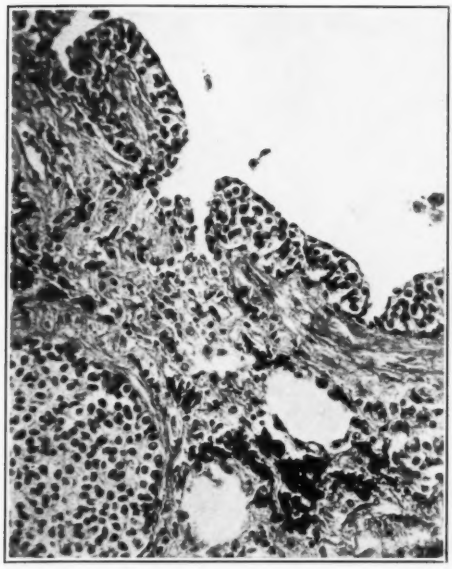
PLATE 118

FIG. 1. Multiplication of bronchiolar epithelium and nodule of small cell carcinoma deeper in the tissue. Hematoxylin and eosin. Green filter, Wratten B 58. × 200.

FIG. 2. Same material as in Fig. 1, stained by the Bielschowsky-Foot method. Note absence of reticulum in the epithelial areas. Orange filter, Wratten G 15. × 200.

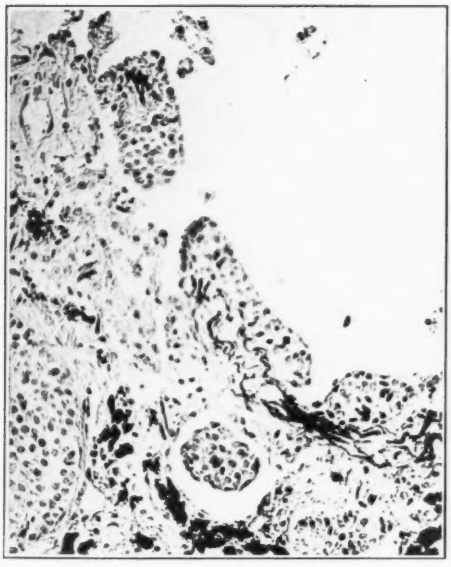
FIG. 3. Same material as Fig. 1, stained by the Verhoeff elastica method. Note the irregular distribution of fibrils, different from that of an artery. Red filter, Wratten A 25. × 200.

FIG. 4. Same material as Fig. 1, stained by the Mallory connective tissue stain. Note the wide spaces between the fibrils in the subepithelial tissue. The spaces are occupied by smooth muscle as demonstrated by the Van Gieson method. Green filter. × 200.

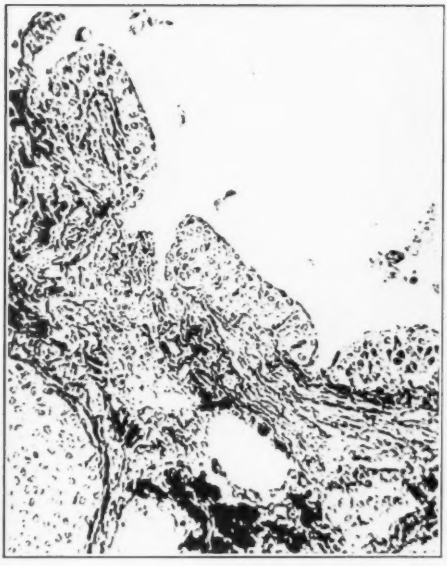


1

2



3



4

Karsner and Saphir Small Cell Carcinomas of the Lung

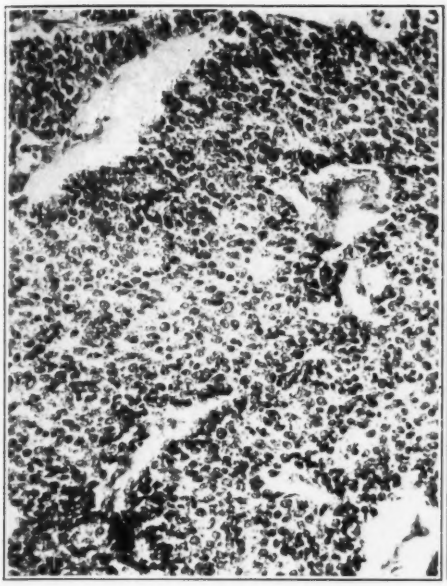
PLATE 119

FIG. 5. Spindle cells and round cells in small cell carcinoma in lung. Note shrunken connective tissue trabeculae. Hematoxylin and eosin. Green filter. × 200.

FIG. 6. Same tumor as in Fig. 5, stained by Bielschowsky-Foot method. Note absence of reticulum in the tumor nodules. Orange filter. × 200.

FIG. 7. Hepatic metastasis of small cell tumor of lung. Hematoxylin and eosin. Green filter. × 200.

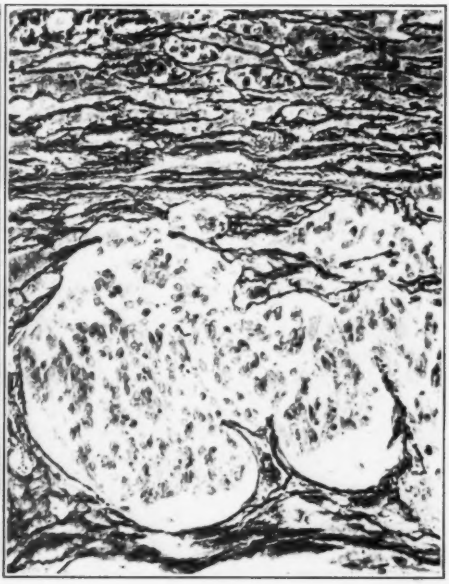
FIG. 8. Area near that shown in Fig. 7 stained by Bielschowsky-Foot method. Note absence of reticulum in tumor nodules. Orange filter. × 200.



5

6

7



8

Karsner and Saphir Small Cell Carcinomas of the Lung

SKELETAL METASTASES IN CARCINOMA OF THE THYROID *

ISAAC LEVIN, M.D.

(From the Cancer Division of the Madison Hospital, New York City.)

Following carcinoma of the prostate and of the breast, carcinoma of the thyroid most frequently metastasizes in the skeleton. Berard and Dunet [1] in their recent analysis on the subject found the frequency of skeletal metastases in carcinoma of the thyroid to vary between 18, 30 and even 70 per cent of all cases. On further analysis of 110 cases of skeletal metastases in carcinoma of the thyroid, they found that the most frequent sites of the metastases were the skull and the vertebrae.

There are several characteristics in the secondary metastatic tumors of carcinoma of the thyroid which make them the most favorable material for the study of the general problem of metastasis formation.

Ewald [2] showed the presence of iodine in metastatic adenocarcinoma of the thyroid while the original tumor was free from iodine.

A simple colloid goiter, presenting a structure that usually remains harmless for years, may give rise to metastatic tumors. These secondary tumors may acquire the characteristics of malignancy though the primary tumor remains benign.

Riedel [3] removed a tumor of the inferior maxilla composed of normal thyroid tissue. The tumor, however, recurred locally after ten years.

In order to elucidate these apparent peculiarities in the formation of metastasis generally, and particularly of skeletal metastasis in carcinoma of the thyroid, a brief survey must be made of the mechanism of formation and pathogenesis of metastasis in carcinoma generally.

The writer [4] studied the problem experimentally and clinically. He also analyzed microscopically a large number of cases of skeletal metastasis. The conclusions reached from this combined study do not always coincide with the opinions of the investigators of purely anatomical material.

* Received for publication May 26, 1930.

Pathologists lay entirely too much stress on the channels of transportation in the formation of metastases generally, and particularly in the predilection of certain types of carcinoma to metastasize in the bone.

Von Recklinghausen,[5] who was the first to investigate the subject thoroughly, maintained that the formation of metastases in the bones is due to the fact that the veins and capillaries of the bone marrow have thin walls, and are not collapsible. These morphological peculiarities favor the accumulation of carcinoma cells within these blood vessels. The majority of pathologists side with von Recklinghausen in the opinion that the mechanical peculiarities are the main, if not the only factors in the causation of skeletal metastases. None the less this hypothesis is inadequate to explain all the phenomena connected with the formation of skeletal metastases. Kaufmann,[6] in a detailed account of a statistical investigation of the frequency of metastases in the bone, found skeletal metastases in carcinoma of the uterus in 3.03 per cent and in carcinoma of the prostate in 66 per cent. The difference between 3.03 per cent of skeletal metastases in carcinoma of the uterus and 66 per cent in carcinoma of the prostate is very striking, and cannot be explained by mechanical differences. The topographical relationships between the uterus and the skeleton on one hand, and the prostate and the skeleton on the other, are practically identical. So is the vascularization of the two organs, and it is as easy for the cancer cells from the uterus as those from the prostate to find lodgment in the bone marrow.

The opinion of Lubarsch[7] that the difference in the relative sizes of the cancer cells and of the minute blood vessels of an organ influence the formation of metastases, is also hardly tenable. The bone marrow contains blood vessels of a sufficiently large caliber to admit the lodgment of a cancer cell of any size.

The only plausible explanation of this difference between tumors of certain organs seems to be that while both the cells of the uterine carcinoma and the carcinoma of the prostate find lodgment in the marrow, the latter cells find there a better soil for development than do cells of the uterine carcinoma. A very striking instance indicating the existence of a peculiar affinity between the cancer cells and bone tissue was presented by Schmorl.[8] He found in a series of cases of carcinoma, metastases not only in the bones of the skeleton, but in bone tissue abnormally developed in the cartilage of the

larynx and trachea, and in one case a metastasis developed in a bone plaque on the wall of the aorta. In the latter case no metastases were formed in any organ with the exception of the bones of the skeleton and of the bone tissue in the wall of the aorta. It is inconceivable that cancer cells should have been able to reach the small bits of bone tissue of the aortic plaque more easily than the parenchymatous organs.

Thus the probabilities are strongly in favor of the conception that tumor emboli are transported everywhere with the same ease and find lodgment in any part or organ of the body. On the other hand, the further growth of the small transported group of cancer cells and their development into a large metastatic tumor depends upon a correct affinity between the cancer cells on the one hand, and the organ cells, the soil as it were, on the other.

The writer has shown in a series of investigations that the development and growth of inoculable malignant tumors of the lower animals depend upon a local interaction between tumor cells and the somatic cells of an organism. In certain instances the parenchymatous cells of a normal organ may inhibit the growth of the tumor, and this inhibition ceases when the parenchymatous cells become degenerated through a certain injury to the organ. On the other hand, the writer has shown that when the cells of a normal organ offer no adequate resistance to the growth of the tumor, then the proliferating tumor cells injure, mechanically or chemically, the former cells. This injury evidently precedes the death of the organ cells and their replacement by tumor cells.

Bone tissue, in view of its characteristic morphological differences from all other tissues, presents a very favorable object for the minute study of the changes taking place in the soil under the influence of tumor growth.

A microscopic study of skeletal metastases of carcinoma which the writer undertook some time ago brought out several points of importance.

The metastasis begins its development within the marrow, and when the group of cancer cells is small, the surrounding bone marrow appears quite normal. Von Recklinghausen claims that the development of the metastasis is preceded by a hyperemia and hemostasis, due to obstruction of the capillaries by the tumor emboli. In the specimens studied by the writer, hemorrhages and hyperemia were

noted frequently around large tumor masses but not around minute metastatic nodules. In the latter instance, as stated above, no morphological abnormality could be found in the bone marrow.

As the tumor nodule increases in size it approaches and invades the compact osseous tissue or the compact osseous partitions of the cancellated bone. Then, there begin to appear the characteristic changes in the bone tissue. It is generally accepted that there are two classes of skeletal metastases of carcinoma: *osteoplastic*, in which extensive new bone formation takes place around the metastatic tumor; and *osteoporotic*, in which the changes in the normal tissue surrounding the metastasis consist in extensive destruction of the compact bone. These two distinct conditions can be easily differentiated on the gross inspection of skeletal metastases. However, the microscopic study of the cases analyzed by the writer showed that both conditions were generally present side by side. The differences in the gross appearance are due to the fact that in one case osteosclerosis or new bone formation predominates, while in another osteoporosis or the destruction of the old bone is mostly in evidence.

The mechanism of the bone destruction in metastases of carcinoma differs from the one observed in inflammatory osteoporosis. Von Recklinghausen first made the observation that the large polynuclear osteoclasts, which destroy the bone in osteoporosis, are very seldom found in the lacunae of the bone surrounding a growing metastasis of carcinoma. This fact was confirmed by most of the subsequent investigators. In view of the absence of the large osteoclasts, von Recklinghausen presumed that there takes place in this bone a softening by the removal of its inorganic salts and a subsequent absorption without the aid of any cells, a condition similar to the one found in osteomalacia. Apolant,[9] Erbslöh[10] and Askanazy[11] are also of the opinion that osteoporosis in skeletal carcinoma may take place without the aid of special cells. On the other hand, Goetsch[12] believes that the cancer cells act as osteoclasts and destroy the compact bone, and Axhausen maintains that the small elongated mononuclear connective tissue cells frequently found close to the walls of the lacunae are special osteoclasts derived from the cancer stroma. In the specimens studied by the writer both cancer cells and the small connective tissue stroma cells were found in immediate apposition to the walls of the lacunae. The cancer cells were so frequently the only cellular elements within the lacunae of the bone

that there cannot be any doubt but that cancer cells do act as direct osteoclasts. The impression gained by the writer is that the small stroma cells only subsequently invade between the bone and the cancer cells. But even if the possibility be admitted that occasionally the stroma cells may act as osteoclasts, it seems quite apparent that the tumor itself, by the aid of its formed elements, first destroys the bone, and then grows by occupying the space so formed.

The mechanism of osteoporosis in skeletal metastases is similar to the mechanism of the destruction of any normal tissue surrounding a growing malignant tumor; the osteosclerosis or the extensive formation of new bone tissue is similar to stroma formation within and around a metastatic tumor.

Von Recklinghausen claims that this extensive new bone formation is due to the hyperemia described by him and mentioned above. Askanazy thinks that the metastasis at first produces an osteoporosis which is followed by a bone necrosis. The necrotic bone acting as a foreign body causes a new bone formation. Wolff,[13] and Kaufmann are of the opinion that cancer cells may act directly as osteoblasts and form new bone tissue.

The mechanism of the new bone formation as observed by the writer is as follows: At first, collagen fibrils are formed in abundance from the old bone. These fibrils gradually unite in thick bundles and subsequently form new bone tissue. The latter is clearly formed from the constituent parts of the old bone tissue. On the other hand, as stated above, the writer did not observe any hyperemia, inflammation, or any other abnormality of the bone marrow at the beginning of the development of the metastases. Furthermore, necrosis of the bone was not found in any of the specimens examined. It must be concluded that some unknown chemical irritant emanating from the cancer cells acts on the old bone tissue and stimulates its proliferation.

Thus two processes always take place side by side in skeletal metastases. On the one hand, the tumor destroys the normal bone tissue, on the other hand the remaining osseous tissue proliferates and creates new bone. The latter is quite probably an attempt at self defense. The new bone tissue may compress and destroy the cancer cells.

The minute microscopic study of skeletal metastases gives the clearest evidence of the interaction which always takes place be-

tween the normal organ tissue and the cancer cells during the beginning of the development of a metastatic tumor from a transported cancer embolus. Upon the result of this interaction depends the success of metastasis formation.

This conception of the pathogenesis of formation of metastases may well serve to clarify the meaning of the peculiarities encountered in the metastases in carcinoma of the thyroid. It is impossible to conceive that a normal cell of the thyroid or a cell of a benign goiter when transplanted into a bone may form a malignant tumor. On the other hand, it is often difficult to differentiate morphologically between a benign and a malignant tumor of the thyroid. It was stated above that the failure or success of the development of a metastatic tumor from a transported embolus depends upon the interaction between the normal organ tissue and the cancer cells.

We may conceive that a small group of cells of a normal thyroid or of a benign goiter may become malignant, but the thyroid or goiter offers an unfavorable soil and suppresses the further development of a malignant tumor for a longer or shorter period of time. The same small group of malignant cells when severed from the primary locus and transported to bone may find there a more favorable soil and develop into a large, actively growing metastatic carcinoma. Moreover these cancer cells may not only increase its power of proliferation but may also increase its functional capacity. Thus Ewald noticed that iodine is produced in the metastatic tumor while none was produced in the primary tumor.

Cases in which the primary tumor in the thyroid is small and not malignant clinically, while the metastatic tumor is large and very highly malignant, are encountered frequently in carcinoma of the thyroid.

This is not only of theoretical, but also of great practical importance. The whole symptom complex is caused by the metastatic tumor, therefore the primary condition is frequently overlooked and both the diagnosis and therapy may be wrong.

The cases of skeletal metastases in carcinoma of the thyroid reported here all belong to this type.

Case Reports

Case 1. *Clinical History:* M. B., white, male, aged 31 years, was seen at the clinic in 1924, suffering from epithelioma of the ear. He disappeared and presented himself again three years later.

At this time the original condition seemed to be improving. About nine months ago an insidious painless swelling arose over the region of the right clavicle. This condition grew slowly but gave the patient no discomfort. Three months later, the patient began to complain of cramp-like pain in the spine. This pain occurred in the daytime, usually at the onset, and was brought on by change of position. In addition to this, the patient complained of pain in the upper spine between the shoulder blades. During this time there was also noted some loss of weight.

Two months later the patient noticed a lump in the occipital region. Other lumps arose in the region of the scalp.

About five weeks ago the patient noticed shooting pains in the legs, originating in the spine. Gradually, power in the lower extremities began to disappear, first on the left side and later on the right.

Physical Examination: General appearance was that of a young adult male about 30 years of age. He presented an anxious expression and appeared to be suffering from recurring pains. He appeared anemic and cachectic.

The right ear was distorted and much smaller than the left. The skin behind the ear was depressed, soft and smooth. The external auditory meatus was occluded by a flat ulcerated neoplastic area 12 by 8 mm. in extent. This area bled readily to the touch and was pinkish in color and granulation-like in appearance.

The chest showed a distinct flattening anteriorly in the region of the nipples. Clavicular regions showed an asymmetry. The right clavicle was much more prominent. On palpation the clavicle was replaced by a soft and elastic, smooth mass about 6 by 3.5 cm. in extent. On palpation the left clavicle also presented many protuberances and depressions. On palpation of the chest, several protuberances were felt.

Over the region of the anterior mediastinum, percussion revealed an area of dullness reaching about an inch to right and left of the sternal margins.

Over the regions of the third, fourth and fifth dorsal vertebrae there was a protrusion and widening of the spine. A similar deformity was felt over the lower lumbar spine and sacrum. The right humerus contained many nodules over its surface as did the left, but to a lesser degree. Both femora presented bony irregularities, particularly in the upper portions.

The patient died on April 26, 1927 and no autopsy was obtained.

Diagnosis: The clinical analysis of the case shows that the polypoid-like structure in the external ear which appeared like exuberant neoplastic tissue was apparently not the epidermoid cancer of skin with which the patient suffered three years ago, but was metastatic in nature. The dull area of the anterior chest began about 1 inch to the right and ended 1 inch to the left of the sternum. In the upper margin it began at the tip of the sternum and went down for 2 inches. The position of the dullness showed definitely that the tumor mass was situated neither in the mediastinum nor in the lungs, but in the intrathoracic part of the neck and consequently represented a *carcinoma of an intrathoracic thyroid, with multiple osteoporotic metastases in the skeleton.* The neurological condition was second-

ary to metastatic carcinoma of vertebrae (paraplegia dolorosa Charcot).

CASE 2. *Clinical History:* A. K., white, male, aged 73 years. In September 1928, the patient developed an acute swelling in and around the left sternoclavicular junction. A few days later a similar swelling developed on the other side and both swellings disappeared in a short time without treatment. A few days later, another swelling appeared in what seemed to be the region of the right lobe of the thyroid. Later, a similar condition appeared on the other side. All the swellings disappeared in a short time. In May, 1929, the patient began to suffer from constant pain in front and back of chest accompanied by coughing and bloody expectoration and also by a high temperature. This acute condition subsided in about ten days. During this period, swellings appeared in the cervical region. About four weeks ago, the patient suddenly felt pressure pain in the back region immediately below the angle of the scapula. This pain continued up to the present. Occasionally, this condition came in spasms, with comparative comfort in between. At present, the greatest pain he has had is in a recumbent position. Patient did not suffer from constipation, had normal bladder function and a fair appetite.

Physical Examination: The patient was emaciated, showing evidence of cachexia and had the appearance of severe muscular tension. In the right supraclavicular region a few lymph nodes were palpated. There was a lymph node noted in the region of the mastoid which was very firm and measured 2.5 cm. in diameter. Another was palpated above the sternocleidomastoid region, which measured 4 cm. in diameter, was hard and nodular, and firmly attached to the underlying structure. There were other lymph nodes noted in both axilla, larger in the left. There were some small inguinal lymph nodes, more on the right side. No other abnormalities were noted.

Results of an X-ray examination of chest and skeleton showed the following conditions:

There was an osteoporosis and decalcification observed in the ribs, spine, pelvis and upper ends of the femora. In addition, there appeared an irregular, destructive process in the superior surface of the second lumbar vertebra with a definite upward concavity.

In the right lung there was noted an irregular infiltrative and fibrotic involvement, especially in the upper lobe, with some bronchiectatic and interstitial pneumonic changes appearing at the right base. Interlobar plastic pleurisy appeared at the level of the fourth rib anteriorly. In the left side, the lung showed a similar infiltrative change in the upper lobe. In addition, at the level of the third rib, there appeared a small circular shadow of increased density measuring about 1 cm. in diameter. A similar nodule about 2.5 cm. in diameter was noted at the level of the sixth rib anteriorly.

Diagnosis: Polyadenitis noted in this case with a history of swellings in the neck appearing and disappearing without treatment is a symptom complex which would indicate that we were dealing with lymphoma malignum (Hodgkin's disease). The two discrete metastatic nodules noted in the X-ray examination may also have been lymphomatous nodules, but the multiple conditions in the skeleton

did not coincide with Hodgkin's disease and a biopsy of the nodule which was situated in the right lobe of the thyroid microscopically showed it to be a papillary adenocarcinoma of the thyroid.

In view of the pathological findings, the conclusion was reached that the symptom complex, particularly the severe pain in the back which was the major complaint of the patient, was due to the carcinoma of the second lumbar vertebra, secondary to papillary adenocarcinoma of the thyroid.

CASE 3. *Clinical History:* White female, aged 38 years. The patient was admitted to the clinic in February, 1927, complaining of pain in the lower part of the back, which radiated mainly to the left thigh. The pain had continued for four months. Patient also complained of irregular menstruation which had continued for the last three years. She was referred to the clinic after a diagnosis of carcinoma of the cervix was made outside the hospital.

Physical Examination: Patient appeared to be well nourished. No adenopathy noted. Thyroid appeared enlarged but seemingly encapsulated and freely movable throughout. Examination of the pelvic organs showed no malignancy in the genito-urinary organs.

X-ray analysis showed an area of irregular rarefaction on the left side of the sacrum involving the first and second sacral segments. The neurological examination seemed to point to a tumor in the lower spine. Subsequent examination of pelvic bones showed that rarefaction was gradually increasing in extent.

Diagnosis: The problem to be solved was whether the rarefaction shown by X-rays was due to a primary sarcoma of the sacrum or metastatic carcinoma with primary seat located elsewhere. It seemed rather unusual for sarcoma of the sacrum to continue for so many months, or for a tumor mass to grow out of the shell of the sacrum and produce a palpable mass.

Examination showed no malignancy anywhere. The only pathological condition encountered was a small, freely movable goiter. Since this goiter may have been the seat of malignancy and if so would cause an additional severe symptom complex if allowed to develop further, a partial thyroidectomy was done and microscopic examination showed it to be an adenocarcinoma of the thyroid.

Subsequently, in an attempt to arrest the rapid growth of the sacral tumor and also in order to control severe pain, a partial excision with insertion of radium was done. Microscopic analysis of the tissue removed showed the identical picture of the thyroid carcinoma. The condition grew worse, notwithstanding all therapy, and the patient died.

Summary

1. Three cases of metastases in the skeleton secondary to carcinoma of the thyroid are reported.

2. In all three cases the clinical symptom complex as well as the evident pathology was caused by tumors in the bone. While the primary tumor in the thyroid was insignificant, both pathologically as well as clinically, compared with the condition in the skeleton, as a result in all three cases, the primary condition was overlooked.

3. Whenever a diagnosis of malignant tumor in the skeleton, single or multiple, is made, a search must be undertaken for a primary malignant tumor elsewhere before the diagnosis of a primary malignant tumor in the skeleton can be made.

4. In such a search for a primary malignant tumor, in a female, next to the breast, the thyroid must be thought of, and in a male, next to the prostate, the thyroid must be considered as the most probable seat of a primary tumor. Such a diagnostic analysis is of importance not only from the theoretical but also from the practical clinical standpoint.

REFERENCES

1. Berard, L., and Dunet, C. Le Cancer Thyroïdien. Paris, 1924.
2. Ewald, K. *Wien. klin. Wchnschr.*, 1896, **9**, 186.
3. Riedel. Berlin Chir-Congressber., 1893.
4. Levin, I. *J. Exper. Med.*, 1911, **13**, 604; 1912, **15**, 163; 1912, **16**, 149; *Proc. New York Path. Soc.*, 1915, **16**, 8; *Ann. Surg.*, 1917, **65**, 326; *J. Nerv. & Ment. Dis.*, 1917, **45**, 481.
5. von Recklinghausen. Festschrift f. R. Virchow, Stuttgart, 1891.
6. Kaufmann, E. Lehrbuch der speziellen pathologischen Anatomie für Studierende und Ärzte. Berlin, 1911.
7. Lubarsch, O. Die Allgemeine Pathologie, 1905.
8. Schmorl. *Verhandl. d. deutsch. path. Gesellsch.*, 1908, **12**, 89.
9. Apolant. *Virchows Arch. f. path. Anat.*, 1893, **131**, 40.
10. Erbslöh. *Virchows Arch. f. path. Anat.*, 1901, **163**, 20.
11. Askanazy. Festschr. f. M. Jaffé, 1902.
12. Goetsch, W. *Beitr. z. path. Anat. u. z. allg. Pathol.*, 1906, **39**, 218.
13. Wolff, J. *Berl. klin. Wchnschr.*, 1869, **6**, 492.

MULTIPLE GUMMAS OF THE HEART IN THE NEW BORN *

JOHN W. WILLIAMS, M.D.

(From the Department of Pathology, Tulane University School of Medicine, New Orleans, La.)

A search of the literature reveals a limited number of cases of syphilis of the heart in the new born that are characterized by the lesion known as gumma. In the majority of the cases reported there are lesions of syphilis other than in the heart. Virchow,[1] in 1858 is probably the first to study lesions similar to the one herein described, which he terms foci of syphilitic interstitial myocarditis. Le Count [2] in 1898, in citing a case of congenital syphilis of the heart in a patient who died shortly after birth, says: "The title of gummata is simply a matter of preference; multiple foci of interstitial myocarditis with leucocytic invasion, areas of degeneration of the heart muscle and the occurrence of multinuclear cells resulted in areas which to the naked eye were limited in extent, whitish, and appeared softened, in other words, gummatous." Hektoen,[3] Adler,[4] Parkinson,[5] and Blacklock and M'Cluskie [6] describe similar cases. Warthin [7, 8, 9, 10] in his publications discusses syphilis of the heart in detail and emphasizes the importance of careful examination in congenital syphilis, citing cases in which the syphilitic involvement was limited to the heart and occurred in no other parts of the body. In a later paper he states: "This form of syphilitic disease of the heart is an important cause of asphyxia neonatorum and unexplained sudden death in early life." The syphilitic heart lesion which I am reporting is similar to the one Warthin [8] terms "myxogumma" and is interesting because there is no other evidence of syphilis in the body.

CASE REPORT

Clinical History: The patient was a full term, negro female infant, aged 3 hours. Delivery was by breach; however, it was not difficult nor was there injury to mother or child. Since the condition of the child at birth was poor, incubation was resorted to and oxygen inhalations given. The mother's Wasserman was strongly positive, but she gave no history of previous illness. She had been pregnant once before and was delivered of a healthy child.

* Received for publication May 16, 1930.

Autopsy Report: The body is that of a well developed and well nourished negro child, 51 cm. in length and weighing 2500 gm. The skin and mucous membranes are clear, moist and elastic and show no signs of desquamation, eruption or fissures. There is no glandular adenopathy or indication of birth injury. The eyes, ears, nose and throat are normal. The chest shows no deformation. The abdomen is not distended. From the recently ligated umbilical cord there is no sign of hemorrhage. The external genitalia, anus and extremities are normal. The peritoneum and pleura are clean and glistening; neither peritoneal nor pleural cavities contain exudate, transudate or adhesions. The thymus and thyroid are normal. The lungs are of normal size, color and consistency. The spleen is slightly congested. The intestinal tract, pancreas, adrenals, bladder and lymphatic system are normal. Grossly, the epiphyses of the long bones show no syphilitic osteochondritis. In the pericardial cavity are approximately 5 cc. of slightly blood-tinged fluid but no indications of acute or chronic inflammation. The epicardium and coronaries appear normal, and on removal the heart weighs 25 gm. The pulmonary artery contains a small postmortem clot. The heart walls are firm, of good color and consistency and in the heart cavities are small amounts of coagulated blood. The foramen ovale is closed and the valves, chordae tendineae and endocardium appear normal. On section of the left ventricular wall, an oval, grayish white nodule measuring 1.25 by 0.5 by 0.5 cm. is seen, which extends from just below the epicardium to the endocardial lining and is situated adjacent to the interventricular septum on the anterior aspect of the heart. Two similar nodules 0.5 cm. in diameter are noted adjacent to the interventricular septum midway between the apex and the auricular ventricular partition, one in the anterior wall of the right ventricle and the other in its posterior wall. These nodules are firm, swollen, well demarcated, protrude slightly and reveal a small area of softening at their centers.

Histopathology: Sections of lesions of the heart fixed in 10 per cent formalin and stained by Levaditi's method reveal countless spirochetes peppered throughout. In the accompanying figure some of the treponema are seen in focus while others appear as black specks. They are especially abundant in the intercellular spaces, around the blood vessels, and in places appear incorporated in the heart muscle fibers.

Shrunken muscle fibers, prominently striated, are seen in sections stained with hematoyxlin-eosin after Zenker fixation. In areas, varying amounts of a mucoid and vacuolated substance separate the muscle fibers. This substance has the appearance of débris and contains bits of striated muscle fiber, lymphocytes, plasma cells, monocytes and polymorphonuclear leucocytes and impresses one with the fact that muscle tissue which might once have been present had been dissolved and its place partially filled with the cells mentioned above. Other areas of muscle fibers show considerable fatty degeneration. Around the small arterioles is seen proliferation of the adventitia and infiltration with lymphocytes, plasma cells and neutrophiles. There is proliferation of the subepicardial tissues and infiltration with lymphocytes, plasma cells, monocytes and polymorphonuclears. Considerable congestion is noted in all areas examined and the capillaries appear extremely numerous. No endarteritis is found and no multinuclear cells are apparent.

Microscopic examination of the liver, spleen, kidneys, suprarenals, lymph nodes and lungs shows no evidence of syphilitic lesions.

Discussion

It is interesting to note that only a limited number of congenital syphilitic heart cases have been reported which have the gross appearance of gumma but whose lesions, studied microscopically, fail to substantiate this diagnosis. Probably not more than twenty cases of this type have appeared in the literature. The similarity of location of most of the lesions is an outstanding factor. They occur adjacent to the interventricular septum in regions supplied by small branches of the posterior descending branch of the right coronary, and of the anterior descending branch of the left coronary artery. This leads one to believe that involvement of such a small branch is responsible for the location of the lesion in the musculature and that the mechanics of the coronaries can explain the branches involved.

The lack of a definite category in which to place this lesion is also of interest. Warthin prefers to call it "myxogumma." The term is somewhat vague since so little is known of the chemical nature of mucins and muscle proteins and since, in any extensive lesion of the heart in which blood, thrombi and fibroblastic proliferations occur, the mucin reaction is apt to be obtained.

In the case of the heart, we are dealing with an organ whose metabolic activities are great and whose cells are highly specialized. In the event that a small infected thrombus blocks a terminal branch of one of its coronaries and a severe inflammatory reaction results, the veins through drainage of the blocked-off area decrease the tissue tension of the heart muscle while the generation of toxic material with its resultant inflammation causes an increased tension. This augments the vulnerability of muscle cells to the effect of toxins and to the action of proteolytic ferments elaborated from the dead cells of the acute reaction. The result is a lesion similar to the one seen in this case. The bits of worm-eaten, dimly striated muscle fibers with nuclei intact and the accompanying cellular reaction are proof of its acuteness and of the enzymatic nature of the destruction wrought. It is therefore more correct to regard this lesion as one of localized syphilitic cellulitis and fulminative syphilitic myositis. This terminology is preferable to that of "gumma" since cellulitis and myositis accurately designate the character of the lesion. Gumma, as we understand it, is the broken-down and encapsulated syphilitic lesion. While it is possible to have true gummas of the heart muscle, none of the so-called cases reported in the new born and herein cited conforms microscopically to our conception of the gumma.

Summary

1. A case of gumma of the heart in a negro infant that died a few hours after birth is reported.

2. The term gumma as descriptive of the lesion is questioned since the microscopic picture is at variance with that of a gumma.

3. The terms localized syphilitic cellulitis and fulminative syphilitic myositis are suggested in its stead as descriptive of this lesion since it is characterized by muscle destruction and infiltration with lymphocytes, polymorphonuclear leucocytes, monocytes and plasma cells.

REFERENCES

1. Virchow, Rudolph. *Virchows Arch. f. path. Anat.*, 1858, **15**, 217.
2. Le Count, E. R. *J. A. M. A.*, 1898, **30**, 181.
3. Hektoen, Ludwig. *J. Path. & Bact.*, 1894–96, **3**, 472.
4. Adler, I. *New York M. J.*, 1898, **68**, 577.
5. Parkinson, J. P. *Rep. Soc. Study Dis. Child.*, 1900–01, **1**, 141.
6. Blacklock, J. W. S., and M'Cluskie, J. A. W. *J. Path. & Bact.*, 1928, **31**, 586.
7. Warthin, A. S. *Am. J. M. Sc.*, 1911, **141**, 398.
8. Warthin, A. S. *J. Infect. Dis.*, 1916, **19**, 138.
9. Warthin, A. S. *Am. J. Syph.*, 1918, **2**, 425.
10. Warthin, A. S. Proc. Internat. Assemb. Inter-State Post-Grad. M.A. of North America (1928), 1929, 511–518.

DESCRIPTION OF PLATE

PLATE 120

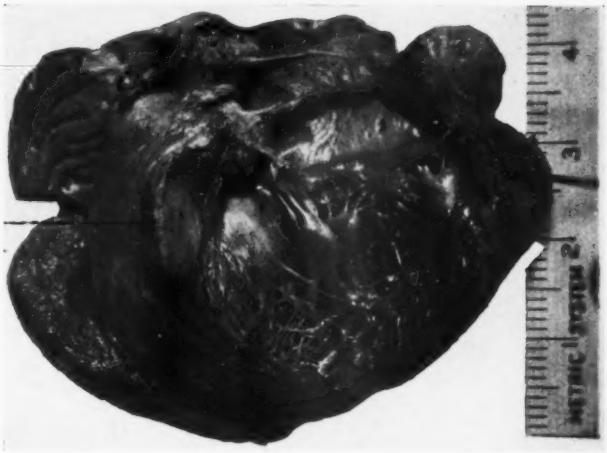
FIG. 1. Enlarged photograph of heart. Note circumscribed syphilitic nodule in anterior wall of left ventricle adjacent to septum with area of softening in center appearing slightly darker.

FIG. 2. Section of heart muscle stained by the Levaditi method. Note innumerable spirochetes diffusely scattered throughout the lesion.

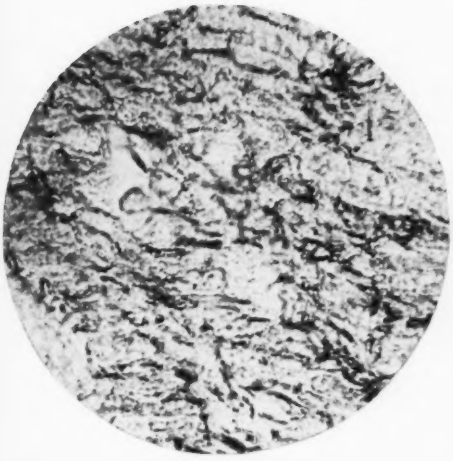
FIG. 3. Section of heart, Zenker fixation, hematoyxlin-eosin stain. Note translucent, vacuolated areas of fatty degeneration of muscle fibers, separation of individual fibers and lymphocytic infiltration around blood vessels and between muscle fibers.

FIG. 4. Section of heart, Zenker fixation, hematoyxlin-eosin stain. Note shrunken, well striated muscle fibers separated by infiltration of lymphocytes, plasma cells, monocytes and polymorphonuclear leucocytes.

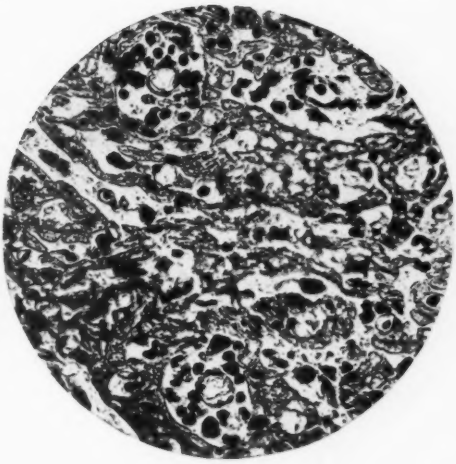
FIG. 5. Section of heart, Zenker fixation, hematoxylin-eosin stain. Note large area with lymphocytes, monocytes, plasma cells, neutrophiles and degenerated muscle fibers with nuclei.



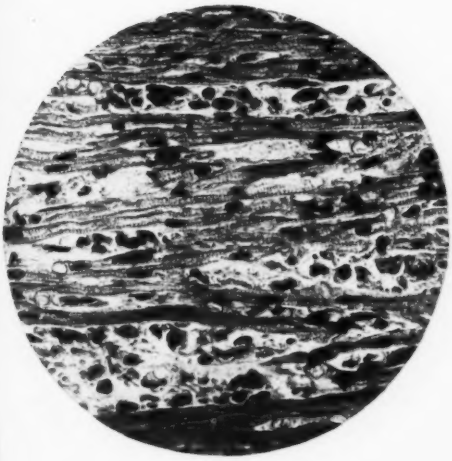
1



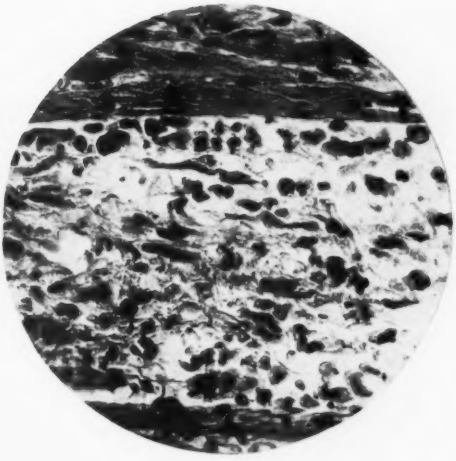
2



3



4



5

Williams

Multiple Gummas of Heart in New Born

SCIENTIFIC PROCEEDINGS OF THE THIRTIETH ANNUAL MEETING OF THE AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

New York City

April 17 and 18, 1930

ABSTRACT OF BUSINESS SESSION
AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

Voted to elect the following officers:

President	GEORGE R. CALLENDER
Vice-President	WARD J. MACNEAL
Treasurer	FRANK B. MALLORY
Secretary	HOWARD T. KARSNER
Incoming Member of Council	OSWALD T. AVERY
Assistant Secretary	ROBERT A. MOORE

ABSTRACT OF MEETING OF THE COUNCIL
AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

Voted to elect the following new members: Nicholas M. Alter, Louis Berger, Zera E. Bolin, Charles F. Branch, W. W. Brandes, Carl J. Bucher, Rafael Dominguez, Norbert Enzer, Hamilton R. Fishback, Jacob Furth, Samuel H. Gray, Robert G. Green, Francis D. Gunn, Ernest M. Hall, Kiyoshi Hosoi, N. Paul Hudson, Wilhelm C. Hueper, Benjamin S. Kline, G. Kenneth Mallory, Jessie Marmorston-Gottesman, Leone McGregor, Valy Menkin, Aura J. Miller, Nickolas W. Popoff, William A. Starin, Bindo deVecchi, Cecil Watson, Charles E. Woodruff, and Arthur W. Wright.

Voted to accept the resignations of Drs. Katharine R. Collins, W. G. MacCallum, Joseph H. Pratt, James S. Simmons, and Paul G. Wooley.

Voted to record with regret the deaths of

Dr. Henry Albert
Dr. Claribel Cone
Dr. Lydia M. DeWitt
Dr. S. H. Gilliland
Dr. Paul A. Lewis
Dr. H. T. Marshall
Dr. Benjamin Roman

The Thirty-first Annual Meeting of the Association will be held at Western Reserve University, Cleveland, Ohio, April 2 and 3, 1931.

The special topic will be "Disease of the Liver — Exclusive of Tumors." Special papers will be presented by Drs. F. B. Mallory and G. H. Whipple.

The Gold Headed Cane of the Association was conferred upon Doctor Theobald Smith for distinguished service to pathology and bacteriology.

AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

FURTHER STUDIES ON THE PRECIPITATION TEST FOR SYPHILIS. Emil Weiss, Chicago, Ill.
(*Abstract not received.*)

THE IDENTITY OF ANIMAL ANAPHYLAXIS AND HUMAN ALLERGY (PROTEIN HYPERSENSITIVENESS). Bret Ratner and (by invitation) Helen L. Gruehl, New York City.

Abstract. In these experiments the blood sera of a bronchial asthmatic sensitive to horse dander, and of guinea pigs sensitized to horse dander by inhalation, were passively transferred to the normal human skin and to normal guinea pigs. Positive passive transfers were obtained both from the transfer of the human serum and of the guinea pig serum. This criss-crossing of the human reagin and of the anaphylactic antibody suggests that both antibodies are identical.

Discussion

(Dr. A. F. Coca, New York City.) It seems much too strong to say that the results obtained by Dr. Ella F. Grove and myself have been refuted by Cooke and Spain. We have independently tested the same sera that Cooke and Spain did (in fact, they obtained all of their sera from us), and we have not been able to obtain this passive transfer to the human skin in order to study it. Also we have given one serum, with which Cooke and Spain obtained positive results, to Dr. M. Walzer, and he has not been able in a single instance to see even a suggested passive sensitization of the human skin. We know that anaphylactic sera will sensitize guinea pigs uniformly, depending on their established sensitizing power. If we find several highly-powered anaphylactic sera which will sensitize guinea pigs regularly, but the human skin very inconstantly, we must conclude that whatever is active in the irregular skin sensitization in these sera cannot be the anaphylactic antibody. On the other hand,the few reported successes in sensitizing guinea pigs with the serum of human asthmatics have been so inconstant that they cannot possibly be referred to the reagens in those sera which will sensitize the human skin constantly, and in a certain dosage with the greatest uniformity. The results are so irregular on both sides that we cannot possibly think these two phenomena can be due to the same substances.

(Dr. Ratner, closing.) I agree with Dr. Coca that the accomplishment of the transfer from the human to the animal, and from the animal to the human skin is rather difficult, and for that reason I believe that a great deal of work should be continued along this line. It is conceivable to imagine that injecting one minim of an animal's serum into a human skin might not have enough of the sensitizing substance to be transferred to such an individual, that particular person being a normally resistant individual. The passive transfer of anaphylaxis from one animal to another is not uniform at all times. We have one instance where one of the animals gave birth to offspring and the offspring were definitely passively sensitized, and we could not take this mother's blood and

sensitize another normal guinea pig. Further, if you could take large amounts of human serum and inject it into normal guinea pigs, you might be able to accomplish this transfer with greater regularity, but you cannot use much more than one or two cubic centimeters. Dr. Grove's remarkable instance of the passive transfer from a human being to a monkey is striking in that she used a very large amount of serum, and the fact that this animal had terrific dyspnea for fifty minutes. This was relieved by adrenalin. This animal was given the same injection three weeks later, and was in a refractory state which Dr. Grove speaks of in a footnote. In the conclusions to the paper she denies the importance of that striking experiment. I believe that there are many factors which we have to clarify, and I certainly do think that instead of criticizing the situation, we ought to continue attempting this transfer, and bring forth further evidence.

SKIN REACTIONS TO THE SOLUBLE TOXIC SUBSTANCE OF THE COLON BACILLUS. Bernhard Steinberg, Toledo, O.

Abstract. Skin reactions to the soluble toxic substance and to the washed, killed bacterial bodies of a twenty-four-hour culture of a colon bacillus were made. Reactions read at the end of twenty-four hours appeared in a large number of normal adults and children who were tested. In people with colon bacillus infections, the reactions to the soluble toxic substance and to the vaccine were either absent or greatly diminished in size and degree. It is assumed that the skin reaction is of an antigen-antibody mechanism. Only occasionally a normal person had agglutinins against the colon bacillus. Immunization with the soluble toxic substance of the colon bacillus reduces the size and degree of the skin reaction against it. The soluble toxic substance has apparently weak antigenic properties.

Discussion

(Dr. E. E. Ecker, Cleveland.) I was very much interested in the work of Dr. Steinberg and congratulate him. There is only one question I should like to ask him, and that is whether he saw any infiltration in these areas, because we found that by repeated intradermal injection with these products into animals we were never able completely to reduce the size of the inflammatory process. There was a reduction in size, but the areas became indurated. It is what Opie calls a local anaphylaxis, and I wonder if Dr. Steinberg has seen it in his human experiments.

(Dr. Steinberg, closing.) I had a personal conversation with Dr. Ecker prior to the meeting, and told him that in some instances we did find slight induration of the skin of these people who presented a negative reaction. I understand that Dr. Ecker is under the impression that he is dealing with an allergic state in some of his animals. I do not know whether I can or cannot subscribe to that opinion yet, in the present state of my work.

THE TYPE DISTRIBUTION OF MENINGOCOCCI IN THE UNITED STATES DURING 1928-1929. Sara E. Branham, Washington, D. C.

(*Abstract not received.*)

IMMUNOLOGIC STUDIES IN BLASTOMYCOSIS. Anna Dean Dulaney, Memphis, Tenn.

(*Abstract not received.*)

PENETRATION OF ANTIBODIES IN THE CENTRAL NERVOUS SYSTEM. Jules Freund, Philadelphia, Pa.

Abstract. Antibodies can be extracted from the brain and spinal cord of rabbits actively or passively immunized with typhoid bacilli.

The titers of the antibodies in the extracts of brain and cord depend upon the titer of the blood serum. In actively immunized rabbits the following numerical relationships exist between the titers of the serum and of these organ extracts: The ration of the titer of the serum is to the titers of extract of brain and of the spinal cord about as 100 is to 0.8; the titer of the serum is to the titer of the cerebrospinal fluid as 100 is to 0.3. In passively immunized rabbits the titer of the serum is to the titer of brain and spinal cord extract as 100 is to 0.7.

The antibodies recovered from the brain are not due to the presence of blood in it, for perfusion of the brain does not reduce its antibody content appreciably.

Antibodies penetrate into the spinal fluid from the blood even in the absence of inflammation of the meninges. When the penetration is completed, the following numerical relationship exists between the titer of the serum and that of the cerebrospinal fluid: 100: 0.25.

The penetration into the cerebrospinal fluid of antibodies injected intravenously proceeds at a slow rate, being completed only several hours after the immune serum has been injected. The penetration of antibodies into the tissue of the brain occurs at a very rapid rate. It is completed within fifteen minutes.

It is very unlikely that when the immune serum is injected intravenously the antibodies reach the brain tissue by way of the cerebrospinal fluid, or that the antibody titer of the cerebrospinal fluid is lower than that of the brain extract, and that antibodies penetrate faster into the tissue of the brain than into the cerebrospinal fluid.

Discussion

(Dr. M. B. Lurie, Philadelphia.) I should like to ask what implications follow from the studies on the distribution of antibodies in the brain and blood of rabbits, in regard to the mode of circulation and formation of cerebrospinal fluid.

(Dr. Freund, closing.) Following intravenous administration of immune serum, antibodies accumulate first in the brain and cord and only later in the spinal fluid. There are more antibodies per gram of organ in the brain and cord than in the spinal fluid (per cc.). On this ground I believe that antibodies found in the brain and cord reach these organs through their blood vessels and not by way of spinal fluid.

Of course these observations have no direct bearing on the question of what would happen if the serum were injected into the lumbar space. Work on this problem is in progress.

ETIOLOGY OF PSITTACOSIS. Charles Krumwiede and (by invitation) Mary McGrath and Carolyn Oldenbusch, New York City.

Abstract. About Jan. 15, 1930, the Department of Health became cognizant that there was illness among persons in contact with sick parrots. The first consideration naturally was whether or not *B. psittacosis* was causing this disease. Of a total of twenty-nine sick or dead parrots received, only one showed the presence of *B. psittacosis*. This parrot had died in a pet shop, and none of the human beings who came in contact with it developed any signs of illness.

Among the human cases of illness there were eleven who had had contact with ill parrots and who finally were considered as having psittacosis. Cultural and serological tests showed no evidence of infection by *B. psittacosis*. The agglutination tests were repeated up to one month after the onset of the disease. These findings seemed definitely to exclude *B. psittacosis* as the etiological agent of the disease.

Fortunately in one instance there became available an ill parrot and two patients who had come in contact with the parrot. Inoculation of parrots with materials from these three cases caused disease and death, and material from these parrots likewise caused death when inoculated into other parrots. The organ emulsions, when filtered through Berkefeld V filters, likewise caused disease and death. Two successive filter passages with the parrot strain and with one of the human strains were successful. These results and the failure to find any common bacterial factor seemed definitely to indicate that the disease, psittacosis, was due to a filterable virus. Mice were found to be susceptible to the virus.

As this demonstration was completed, Bedson, Western and Simpson (London) reported similar findings. Since then, Levinthal (Berlin), Armstrong, McCoy and Branham (Washington) have also demonstrated the presence of a filterable virus.

Discussion

(Dr. F. P. Gay, New York City.) I should like to ask Dr. Krumweide if he has made any observations on chlamydozoa or other bodies of that sort in either animals or human beings.

(Dr. S. E. Branham, Washington.) I should like to emphasize what Dr. Krumwiede said about the absence of Nocard's bacillus. At the Hygienic Laboratory we examined bacteriologically at least twelve birds that had spontaneous psittacosis infection, twelve normal birds, twelve birds experimentally infected, and material from four cases of psittacosis, one fatal, and we did not find, although we picked more than 400 colonies, one single culture of any member of the paratyphoid group of bacteria.

(Dr. R. D. Lillie, Washington.) I have heard Dr. Krumwiede's paper with much interest, and I should like to mention some other findings we have observed in the Hygienic Laboratory which are a little bit aside from the filtrations. I had an opportunity of examining histological tissues on some fourteen parrots and parrakeets, three of which showed lesions. One of these was directly associated with human cases of the disease, and the rest were intimately exposed to material from other parrots that were definitely associated with human cases. These three parrots showed focal coagulation necroses in the liver, and large Kupffer cells, and in one of them there was a proliferative reaction of the pleural endothelium. In all of the three parrots I found very minute coccoid and bipolar bacillary cell inclusions in the Kupffer cells of the liver, in the pleural endothelial cells, and also in the bone marrow. These findings are parallel with those from two human autopsies which I had studied through the kindness of the Department of Health of Philadelphia and of the Naval Medical School in Washington. These human cases also showed the focal necroses in the liver. The major lesion was in the lungs, where there was pneumonia with the exudate largely of large round celled type, and in these two cases I was able to demonstrate after prolonged search the same inclusions in these large round cells, both within the exudate and within the alveolar wall. These cell inclusions measure between 0.2 and 0.3 micron, and I believe they

would be able to pass a filter, if any visible organisms can be said to pass filters (*cf.* U. S. Public Health Reports, **45**, 773, April 11, 1930).

(Dr. Herbert Fox, Philadelphia.) Is there any evidence of difference in the infection of parrots of this group that has come from South America, and previous outbreaks of what has been called psittacosis as far back as the naming of the organisms in 1892? In the past twenty-four years at the Philadelphia Zoölogical Gardens there have been four outbreaks among parrots, to which the term psittacosis may be applied in the sense that there was a pneumonia, a degeneration and infiltration in the liver, and on two occasions, when the attempt was made, organisms of the paratyphoid-paracolon group were isolated. From what I can learn of the cases this time, the picture is not the same. Is there any evidence to indicate that the disease among birds is distinctly different from that in former years, because the psittacosis organism was not difficult to obtain from the previous outbreak?

(Dr. Stuart Mudd, Philadelphia.) There are several other visible microorganisms which can be said surely to pass the filter under proper conditions.

(Dr. Krumwiede, closing.) As regards the disease in parrots, I have no doubt that there is a definite *B. psittacosis* infection in parrots, analogous to the infections we have in laboratory animals, in rodents, in food-producing animals (including ducks), and in man, as Miss Cooper and I, as well as others, have shown.

With the unfortunate illness of Miss McGrath, Miss Oldenbusch and Miss Buzen who assisted, our work was necessarily curtailed. The material available was turned over to Dr. Rivers as he wished to study the virus, also the histology, facilities for which were not immediately available to us.

In regard to Dr. Lillie's important observation on tiny bodies in the tissue, I might add that Levinthal (in a reprint just received) states he observed coccoid and bacillary forms in smears from tissues and filtrates.

A Study of the Effect of Tubercle Bacillus Lipoids on the Tuberculin Reaction. Esmond R. Long and (by invitation) Arthur J. Vorwald, Chicago, Ill.

Abstract. Doses of 0.02 to 0.05 of a milligram of purified soluble tuberculin protein cause an intense interstitial cellular infiltration and profound degeneration of the cells of the spermatic tubules in the testes of tuberculous guinea pigs (tuberculin reaction). Equal doses of purified lipoids in fine colloidal suspension cause a slight interstitial wandering cell infiltration and no degeneration. Mixing tubercle bacillus fatty acid with tuberculin protein enhances the tuberculin action of the latter in that the fluid element of the exudate is greater and the amount of fibrin present is increased. No change in the intensity of cellular infiltration or necrotizing effect on the testicular tubules or spermatocytes has been noted. The increased fluid output suggests that an increased degree of toxic action on the capillary endothelium has occurred. Ultramicroscopic examination has shown no change in the degree of dispersion of the colloidal particles incident to mixing the two colloidal preparations.

The Specific Cytotoxic Action of Tuberculin on Tissue Culture. Joseph D. Aronson, Philadelphia, Pa.

Abstract. The specific cytotoxic action of tuberculins prepared from the various acid-fast bacteria upon the bone marrow and the spleen of tuberculous and of non-tuberculous guinea pigs was studied by means of tissue culture.

It was found that cells from bone marrow and spleen of tuberculous guinea pigs when planted in plasma from tuberculous or non-tuberculous guinea pigs fail to migrate and to multiply in concentrations of tuberculin prepared from either the human or the bovine type of tubercle bacillus, and on the other hand that such concentrations of tuberculin do not interfere with the migration and multiplication of cells from bone marrow or spleen of non-tuberculous guinea pigs. When high dilutions of tuberculin are added to explants of bone marrow or spleen of tuberculous guinea pigs, migration of cells occurs, but the cells are shrunken and rounded, whereas the cells of tissues from the non-tuberculous animals are larger, irregular in outline and vesicular.

Tuberculins prepared from the avian type of tubercle bacillus, from the various acid-fast bacilli found in fish, snakes, frogs and turtles, from several different strains of the lepra bacillus and from a number of different acid-fast saprophytes did not interfere with the migration and multiplication of the cells from explants of bone marrow or spleen of tuberculous or of non-tuberculous guinea pigs. Similarly tuberculoprotein, the phosphatid and the carbohydrate fraction of the human type of tubercle bacillus fail to inhibit the migration and multiplication of the cells from the bone marrow or spleen of tuberculous or of non-tuberculous animals.

The addition of plasma from a tuberculous guinea pig to explants of the bone marrow or spleen of a non-tuberculous guinea pig does not render such explants susceptible to the cytotoxic action of tuberculin prepared from the bovine or human type of tubercle bacillus.

Discussion

(Dr. E. R. Long, Chicago.) Tissue culture experiments on tissues removed from tuberculous animals which are sensitive to tuberculin, always raise this question: How far down the line of descent from the original sensitive cells does the sensitiveness persist? I may say that in the testes of the tuberculous animal, where we can see clearly the line of descent, the spermatocyte is profoundly sensitive to tuberculin, whereas its grandchild, the spermatozoön, is not demonstrably sensitive.

(Dr. Aronson, closing.) Fisher found that fibroblasts which had been grown from tuberculous chickens for several generations were sensitive to the action of avian tuberculin. In my studies I found that the bone marrow cells from the first generation of a tuberculous guinea pig became rounded and shrunken when tuberculin was added.

THE MECHANISM OF THE INFLUENCE WHICH AN INFECTIOUS PROCESS EXERTS ON THE IMMUNITY RESPONSE OF THE ORGANISM. L. Dienes, Asheville, N. C.

(*Abstract not received.*)

PROGRESS IN CHARACTERIZING ANTIBODIES AND ANTIBODY ACTION. Stuart Mudd, Balduin Lucké and (by invitation) Morton McCutcheon and Max Strumia, Philadelphia, Pa.

Abstract. Analysis of the mechanism by which blood serum promotes the phagocytosis of bacteria and other foreign particles has been continued. Sera which promote phagocytosis of bacteria or of red blood cells have been found invariably to cause certain definite changes in the surface properties of their homologous antigens, namely, increased cohesiveness, decreased surface potential

difference and increased resistance to wetting by oil. Similarly, but with certain exceptions, sera which cause these characteristic changes in the antigen surfaces also promote phagocytosis. The phagocytosis-promoting, agglutinating and surface effects are in quantitative correspondence. A similar striking correspondence has been found between the agglutinating, surface and phagocytosis-promoting effects caused by the euglobulin and pseudoglobulin fractions of antibacterial sera.

Following the technique of F. S. Jones, collodion particles were coated with precipitinogen and then treated with homologous precipitin sera and their protein fractions. The same sera and serum globulin fractions cause specific precipitation, agglutination or phagocytosis according to the conditions under which they are allowed to react with their homologous antigens.

Agglutinating, surface and phagocytosis-promoting effects have thus been found to be quantitatively parallel. This quantitative correspondence of the several effects of a serum or serum globulin fraction has been found both for antibacterial and for antiprotein sera and their globulin fractions. All of the effects are consequences of the deposit of sensitizing serum substances on the antigen surface. The surface deposit so produced by maximal sensitization with homologous rabbit antiserum has similar properties whether deposited upon acid-fast bacteria, upon erythrocytes, or upon precipitinogen-coated collodion particles, *i. e., the sensitized surface has wetting properties characteristic of protein, is cohesive, and has an iso-electric point between pH 5.5. and 5.8. These are the properties also found for specific (immune) precipitate.*

These studies thus bring evidence of a new sort in support of the following simple generalization: *The combination of antigen and antibody is determined by specific chemical affinities. The effects following this combination, namely, precipitation, agglutination, changes in surface properties and phagocytosis, are consequences of the properties primarily of the antibody-protein combined with and deposited upon the antigen surface.*

Discussion

(Dr. C. B. Coulter, New York City.) The point which Dr. Mudd has emphasized with regard to the shift of the iso-electric point toward the more alkaline region is I think a rather important one. There are plenty of people who would say that the immune body is a perfectly independent substance and not an essential part of the protein with which it is associated. If that were the case, we should not expect the shift which Dr. Mudd has described. The explanation which was discussed by Dr. Mudd seems to be not wholly satisfactory, that is, the presence of fibrinogen which has itself a more alkaline iso-electric point than that of the euglobulin cannot be the explanation. The data of Dr. Mudd suggest that the immune element of the protein molecule may involve the properties of the molecule as a whole; this is perhaps reflected in the *orientation* of the protein molecule in the process of combining with antigen in the immune reaction.

(Dr. Jules Freund, Philadelphia.) Is it possible to isolate from immune serum obtained from the rabbit a substance that has an iso-electric point of pH 5.7? Dr. Felton isolated a very potent substance from immune horse serum, which has an iso-electric point (minimum solubility) at pH 6.6.

(Dr. Mudd, closing.) I can only agree with Dr. Coulter's remarks. There is one item of evidence I did not stress which points in the direction he has indicated, namely, that the euglobulin, which is one of the active fractions, when

precipitated by dilution and acidification has an iso-electric point at pH 5.1, whereas the precipitin, uniting with the antigen, gives a specific precipitate whose iso-electric point is at pH 5.7 to 5.8. The differences between these two values may be due to altered orientation of the antibody, possibly some of the dissociating groups being combined, or else one of several other possibilities must obtain. We are investigating these questions further.

In regard to Dr. Freund's question, I may say that the difference between the minimum solubility of pH 6.6 and 6.8 which Felton found for his anti-pneumococcus antibodies, and our value of pH 5.6 to 5.8 does offer a very important point of departure for further analysis of what this antibody protein is. Felton and I have been in communication, and intend to go ahead and begin further analysis of this point in the next few days, but I have no other data to offer at the present time.

THE BUCHNER RENAISSANCE IN IMMUNOLOGY. W. H. Manwaring, Stanford University, Calif.

Abstract. There is convincing evidence that injected antigens undergo a series of biochemical "hybridizations" in animal tissues, and suggestive evidence that the resulting antigen-"hybrids" become semipermanently "symbiotic" with these tissues, both terms, of course, being used metaphorically.

There is convincing evidence that some of these antigen-"hybrids" have properties simulating those of specific antibodies, but no proof thus far that they are identical with these antibodies.

The "hybridization" metaphor, however, furnishes a logical and consistent explanation of all known facts of specific immunological adaptation, and is the nearest approach to a complete immunological theory that can be formulated at the present time.

There is no proof at the present time, however, that antigen "hybridization" is the only mechanism of specific immunological adaptation, and no suggestion that such "hybridizations" are not supplemented by non-specific functional mutations and hypertrophies.

Discussion

(Dr. F. P. Gay, New York City.) I should like to speak with enthusiasm of this work of Dr. Manwaring's, as I made experiments in 1912, embodied in two typewritten pages of manuscript which was never published, because I could not repeat the results. I made a mixture of normal horse and rabbit serum, and incubated it, and in one instance I found precipitins on adding horse serum to the mixture, but I was never able to repeat it, and I am glad to find I was probably right, after all.

(Dr. F. M. Huntoon, Glenolden.) I should like to add to Dr. Gay's experience. Once by incubating an extract of pneumococcus Type I with the juice obtained from cabbage, I was able to say later that this mixture contained agglutinins for the pneumococcus. Later I showed that it contained protective antibodies also. Like Dr. Gay, I never published my results, because I spent three months trying to repeat them, and could not do so.

(Dr. Stuart Mudd, Philadelphia.) I think it is only fair to Dr. Arthur Locke to say that he has done the same thing by incubating red blood cells with serum, and by treatment with ether, splitting off hemolysins, and he has published his results.

(Dr. A. F. Coca, New York City.) The theory that antibodies are composed of antigen united with some normal body substance is attractive; however, the theory seems to face serious difficulty because of the long period of time during which antibodies are often produced after inconsiderable antigen injections. It is a well known fact that antibody production can continue, and often does continue throughout life after only a very moderate contact with the antigen, as in the case of antitoxins in what we call the naturally immune person. This could be explained, in a way, under the theory, by assuming a continual contact with the antigen throughout life after the antitoxin has once been formed, but Park's observations seem to have settled that argument very conclusively when he showed that an attempt at active immunization in babies who were still immune because of the transfer of passive immunity from the mother to the child, did not, after a short period, show an acquired active immunity. Just as many of these children become susceptible after this treatment as would have been expected if they had not received the injections. In other words, the antigen contact in the presence of antibodies is not sufficient, under these conditions, to continue the antigenic stimulation. Therefore we still have to explain why antibodies continue to be produced throughout life. Also, we have the facts brought out by Heinbecker's study of the Eskimos, who presumably do not come in contact with diphtheria bacilli at all, and still show an immunity to diphtheria.

These considerations point to antibody production as the secretion of a synthetic body similar to the secretion of enzymes, rather than as an elimination of antigen conjugated with a normal body substance.

THE CYTOCHROME OF BACTERIA. C. B. Coulter (by invitation) and F. Stone (by invitation), New York City.

Abstract. The intracellular pigment cytochrome which is found in all living plant and animal cells except the anaerobic bacteria, was originally observed by McMunn, and was rediscovered and renamed by Keilin. We have studied the occurrence of this pigment, particularly in the corynebacterium group of bacteria. The four absorption bands observed by McMunn and Keilin are found by spectroscopic examination in suspensions of living bacteria of all the members of the group. In addition there is found a band lying in the red portion of the spectrum. Filtrates of cultures show no selective absorption except in the case of toxin-producing diphtheria bacilli, and in filtrates of these the intensity of selective absorption runs parallel with the amount of toxin present. The characteristic absorption spectrum may, therefore, be that of the toxin itself. It has been possible by spectroscopic examination alone of different filtrates, to determine the presence and approximate amount of diphtheria toxin.

RHINOSPORIDIUM SEEBERI: PATHOLOGY AND REPORT OF THIRD NORTH AMERICAN CASE. Carl V. Weller, Ann Arbor, Mich.

Abstract. The clinical findings and histopathology from the third case of *Rhinosporidium seeberi* to be reported from North America are described. The stages of the development of the parasite, as well as the character of the accompanying granulation tissue, are illustrated, as far as is possible. The foreign body giant cell reaction which succeeds the rupture of the old cysts is described for the first time.

Discussion

(Dr. A. S. Warthin, Ann Arbor.) About six years ago I found in the crypts of two tonsils that came in the ordinary diagnostic work in my laboratory, organisms similar to these. At that time I regarded them as simply spore cases of some unknown mold, but since then our attention has been drawn to the *Rhinosporidium*. I believe that they represent *Rhinosporidium*. Dr. Weller thinks it is probably a new species. I sent photomicrographs of these two tonsils to Dr. Talliaferro in Chicago, and he regarded them as *Rhinosporidium seeberi*, but asked to see the sections, which I have not sent him as yet. If they prove to be *Rhinosporidium seeberi* these will make the fourth and fifth cases on record in North America. It is interesting that one of these cases was a young female, which would be the first case in which a female was infected.

CELLULAR IMMUNITY IN ACQUIRED AVIAN MALARIA. P. R. Cannon and (by invitation) W. H. Talliaferro and L. G. Talliaferro, Chicago, Ill.

Abstract. Previous papers in this series by Talliaferro and Talliaferro demonstrated a definite immunity to superinfection in canaries infected with *Plasmodium cathemerium* and indicated that this immunity is not associated with a humoral antibody, inasmuch as serum from birds with latent malarial infections was without both protective and curative action on the development of the parasites in normal birds.

The present paper reports observations on the cellular reactions in the tissues of normal birds and of others superinfected. The tissues were fixed, sectioned and stained with Maximow's hematoxylin-eosin azur II. In the normal birds it was found that phagocytosis begins as soon as parasitized red blood cells are injected, being most pronounced in the spleen and liver, and consisting in the ingestion of the parasite-red cell combination rather than of individual merozoites. In spite of a constant rate of phagocytosis, many parasitized red blood cells escape ingestion and sporulation occurs with extension of the infection. Thus, the process advances until a crisis occurs on approximately the eighth day, after which time the rate of phagocytosis apparently exceeds the rate of reproduction and the infection becomes latent.

In the superinfection, however, the parasitized red blood cells injected are removed from the circulating blood stream extremely rapidly, so that within twenty-four hours there are few survivors to sporulate. This altered reactivity seems to be due to an actually increased number of actively phagocytic cells within the spleen and to a less degree in the liver. Because of this fact, the parasitized red cells are quickly removed from the blood and the superinfection terminated. The mechanism of the immunity then, is an increased rate of phagocytosis by the cells of the reticulo-endothelial system, resulting from an increased number of actively functioning phagocytic cells which are present in birds with latent infections.

THE PROTECTIVE EFFECT OF SPLENIC TRANSPLANTS IN ALBINO RATS AGAINST BARTONELLA MURIS ANEMIA. David Perla and (by invitation) J. Marmorston-Gottesman, New York City.

Abstract. *Bartonella muris* anemia is an infectious disease occurring in albino rats following the removal of the spleen. It may be transmitted into normal young rats and young rabbits by the injection of whole blood from anemic

splenectomized rats and the strain of the virus maintained by repeated passage in young rats.

We have succeeded in isolating a strain of *Bartonella muris* anemia from normal non-splenectomized adult rats by injecting the blood of the anemic rabbit into young rats. This demonstrates the fact that the adult rat is a carrier of the virus *Bartonella muris* anemia. It is found further that splenectomy in suckling rats is not followed by the *Bartonella muris* anemia since during the suckling period the rat is not a carrier of the virus.

Trypanosoma lewisi infection in adult normal rats is accompanied by *Bartonella muris* anemia. The trypanosome infection produces profound changes in the pulp tissue of the spleen. Functional injury to this pulp tissue produces the same effect in the adult albino rat as splenectomy, in lowering the resistance to the *Bartonella* infection.

Less than one-fourth of the spleen left in situ is sufficient to prevent the development of the *Bartonella muris* anemia. Autoplastic transplants of small pieces of splenic tissue made six weeks prior to the removal of the spleen prevent the development of *Bartonella* anemia in over 50 per cent of the rats, whereas transplants made four weeks prior to splenectomy do not protect. Histological examination of the transplants in the animals that were protected from the anemia reveals complete regeneration of all elements of the splenic tissue, both malpighian bodies and pulp cells. In the rats that were unprotected, the splenic transplants showed complete regeneration of the malpighian bodies, but exhaustion destruction of the pulp tissue. This demonstrates that the pulp cells of the spleen are specific in the protective mechanism of the rat to *Bartonella muris* anemia.

Discussion

(Dr. P. R. Cannon, Chicago.) In studying this question a year or so ago, we made somewhat similar observations. In our experience we found that the only rats that did not have this infection were from the Wistar strain. In studying the spleens of these animals and the ones that had the *Bartonella* infection, the difference was clearly one of increased amount of phagocytic tissue in the animals with the *Bartonella* virus, with large splenic follicles rich in mitotic figures in the case of the infected animals, whereas the lymphoid follicles were compact and small in the animals free from infection. We had a similar experience in removing a large proportion of the spleen. As much as three-fourths of the spleen was removed with no anemia resulting: two weeks later, however, the removal of the remaining fourth was followed by the typical so-called *Bartonella* anemia.

AVIAN TUBERCULOSIS IN NORMAL AND VACCINATED RABBITS. E. M. Medlar, Mt. McGregor, N. Y.

Abstract. Virulent avian tubercle bacilli when inoculated subcutaneously into rabbits cause a local abscess which ruptures and heals. The rabbits do not develop generalized tuberculosis and do not succumb to the subcutaneous inoculation.

Rabbits inoculated intravenously with 1 mg. or more of virulent avian tubercle bacilli always succumb to the infection in from two weeks to a month. A study of the histopathology of such animals shows extensive involvement of spleen, liver and bone marrow. The cell chiefly concerned in the reaction is the mononuclear leucocyte or monocyte. Many lesions show slight abscess forma-

tion due to the participation of the neutrophile. The presence of "myeloid" giant cells, which are megalokaryocytes, in the spleen, liver and lungs, suggests a similarity between the process and Hodgkin's disease.

The daily leucocyte records of the animals show a close parallelism with the histopathology found. In practically every instance the leucocyte picture became septic in type a short time before death occurred.

A group of rabbits inoculated six months previously with living human tubercle bacilli were given an intravenous injection of avian tubercle bacilli, the same amount being given to a group of controls on the same diet. The injection of human tubercle bacilli had caused a local abscess which ruptured and healed, as did the subcutaneous inoculation of avian tubercle bacilli. The animals had normal blood counts and were in excellent physical condition at the time of their avian inoculation.

One of these rabbits died within seven days. The remaining animals lived from four to five months. These animals showed marked involvement of kidneys and joints. There were a few tuberculous foci in the lungs and liver. The histopathology was similar to that seen in chronic tuberculosis in human beings.

Daily leucocyte records showed the establishment of a septic leucocyte picture long before death of the animal. This corresponds closely with the leucocyte picture present in chronic progressive human tuberculosis. Here again the leucocyte picture closely parallels the histopathology present.

THE RELATIVE PHAGOCYTIC ABILITY OF MONOCYTES AND LEUCOCYTES. Balduin Lucké, Max Strumia (by invitation), Morton McCutcheon (by invitation), and Stuart Mudd, Philadelphia, Pa.

(*Abstract not received.*)

THE USE AND THE REASONS FOR THE USE OF THIOCRESOL TO STIMULATE WOUND HEALING. Stanley P. Reimann, Philadelphia, Pa.

Abstract. Since Hammett has shown that the sulphydryl group (–SH) is essential for cell division, this group, when presented in proper concentration to cells, should stimulate mitosis. The healing of wounds is essentially a matter of cell proliferation; consequently –SH compounds should hasten healing. The question to which group the –SH radicle should be attached is apparently not of essential importance although it is of practical interest. Of a number of compounds tried, thiocresol proved quite advantageous. Theoretically at least, when this compound is split, the small amount of cresol present tends to inhibit bacterial growth, while not interfering materially with mitosis of the tissue cells. On old wounds, ulcers, and so on, it is used as a wet dressing in a 1:10,000 solution. Certain practical points must be observed, such as the maintenance of proper growth balance between epithelium and granulations, etc.

EXPERIMENTAL STUDIES IN POLIOMYELITIS. Richard Thompson (by invitation), New York City.

Abstract. Efforts to adapt the virus of poliomyelitis to the rabbit organism and to produce poliomyelitis in rabbits by testicular injection, and by brain injection after testicular passage, produced no evidence that the virus could be adapted in this manner. Suggestive symptoms produced in very young rabbits were duplicated in non-specifically treated and in uninoculated controls. The admixture of a vaccine virus adapted to the rabbit organism, with the polio-

myelitis virus in similar injections and passages, did not aid the adaptation. The virus of poliomyelitis, whether alone or mixed with vaccine virus, does not survive twenty-four hours in the rabbit testicle.

No neutralizing substances against poliomyelitis virus could be produced in rabbits by the repeated intraperitoneal and intradermal injection of poliomyelitis virus or of poliomyelitis-vaccinia virus mixtures.

Although attempts to infect monkeys by intrastomachic injections, after bile irritation of the mucosa, were entirely negative, evidence was obtained that repeated intrastomachic injections after bile irritation may produce an appreciable degree of immunity.

No evidence could be obtained that the cellular elements of the blood contain the virus in any greater proportion than the whole blood.

SPECIFIC AND SPECIAL INFLAMMATIONS OF THE NERVOUS SYSTEM. Simon Flexner, New York City.

Abstract. During the past twenty-five years, largely because of the extensive epidemics of meningitis, poliomyelitis, and encephalitis which have prevailed in that period, much attention has been directed to infections of the central nervous organs. At the present time, attention is being concentrated on encephalitis, of the epidemic form or of the variety to which the designation "post-vaccinal and allied conditions" is being applied. The important fact that herpes virus is capable of inducing encephalitis in rabbits, guinea pigs, rats, and mice, and occasionally in monkeys, has come to play a significant part in the supposed etiology of epidemic encephalitis.

The purpose of the present report is to draw attention to the multiple incitants of encephalitis, and hence to distinguish between encephalitis as a clinical-pathological condition and epidemic encephalitis, which appears to be a specific infectious disease. Just as there are multiple incitants for the clinico-anatomical condition to which the term "diphtheritis" is applied, and a specific incitant of the disease diphtheria, so, probably, there are also many sources of encephalitis and, probably, a single etiological source of epidemic encephalitis. If this view is correct, then the mere production of encephalitis in animals does not imply the experimental reproduction of epidemic encephalitis.

PRODUCTION OF GLOMERULONEPHRITIS IN RABBITS BY STREPTOCOCCUS HEMOLYTICUS. Maud L. Menten, Pittsburgh, Pa.

Abstract. Four strains of *Streptococcus hemolyticus*, two of which belonged to the *Streptococcus scarlatinae* group, were used. Intravenous injections of 0.5 to 1 cc. of filtrates of the above, produced degenerative changes in the endothelium of the tuft. A few of the glomeruli were pale and bloodless, but the majority were congested and the endothelium was vacuolated. Injections of 5 to 10 cc., or larger amounts of the toxin precipitated from the filtrate, caused widespread, marked degeneration of glomerular endothelium and granular degeneration of tubular epithelium accompanied by varying amounts of hemorrhage into the capsular spaces.

Repeated injections of the filtrate (0.5 to 1 cc.) resulted in destruction and disappearance of many secretory units. In a few cases as many as 50 per cent of the glomeruli had disappeared when the animal was killed. Remaining glomeruli showed swelling, congestion and vacuolation of the endothelium.

Repeated injections (1 cc.) of suspensions of live organisms gave similar results but of much less intensity.

Four or more weekly or biweekly injections of filtrate followed by injections of live organisms caused in addition to the above-mentioned degenerative lesions, acute glomerulitis, focal infiltrations of lymphocytes, especially in areas where previous tubular degeneration had occurred, and scattered patches of early fibrosis with compensatory dilatation of the tubules. In 15 per cent of the animals, glomerular nephritis with characteristic epithelial crescents, early fibrosis of tufts and pericapsular fibrosis were also observed. Preliminary alteration due to toxin apparently renders the glomeruli more susceptible to action of live organisms with a resulting production of chronic glomerular lesions.

THE RELATION OF THE TYPE OF RENAL EPITHELIAL REPAIR AND RENAL FUNCTION TO THE NUMBER AND TYPE OF CASTS IN THE URINE. Wm. DeB. MacNider, Chapel Hill, N. C.

(*Abstract not received.*)

CHRONIC FOCI OF INFECTION EXPERIMENTALLY PRODUCED. Virgil H. Moon, Philadelphia. Pa.

Abstract. The usual methods of animal inoculation are not suited to the study of chronic focal infections. They do not approximate the conditions present in human diseases. A new method for producing foci of infection in animals is reported.

Applicators of cotton are loosely wound on No. 20 or No. 22 rustless wire so that slight force will slip the swab from the wire. A trocar and canula are inserted into the region to be inoculated, the trocar is withdrawn, leaving the canula in place. The applicator is dipped into the material to be inoculated and is inserted through the canula. The swab is pushed quite beyond the end of the canula. Then, upon withdrawing the wire, the end of the canula pushes off the cotton into the tissue or body cavity. The canula is then withdrawn.

The method is a simplified procedure for implanting infection in a porous substance within the tissues. The foreign substance serves to maintain the infection against effective body resistance. The method is well suited for producing chronic foci in subcutaneous and intramuscular areas and in the body cavities. By exposing the viscera, infection may be implanted in the spleen, liver, gall bladder or other structures. In such instances smaller canulas and correspondingly smaller applicators are used.

Dogs, which are so resistant to ordinary inoculations that they are seldom used for the purpose, are easily infected by this method. When inoculated with streptococci, masses of infected granulation tissue 3 to 5 cm. in diameter develop in a few weeks. The inoculated organisms are regularly recovered from the inoculated focus and frequently from the substance of other organs. Streptococci have been recovered in pure culture six to ten months after implantation.

Only young dogs and rabbits have been used in these experiments. The pathological changes resulting from these chronic foci of infection include verrucose endocarditis with rheumatic nodules in the heart musculature, skeletal muscles and subcutaneous tissue, acute vegetative endocarditis with multiple infarctions, periarthritis, osteo-arthritis with anklyosis and deformity, nephritis of various forms, splenic atrophy and fibrosis, proliferative endarteritis and others.

FURTHER STUDIES ON THE CERVIX UTERI AS A FOCUS OF INFECTION IN CHRONIC ARTHRITIS. L. W. Famulener and (by invitation) Frederic J. Matthews, New York City.

Abstract. About six years ago we began our studies on chronic arthritis from the etiological and the therapeutic standpoint. The chronic inflammatory condition exhibited by this disease would indicate that it is probably induced by an irritant, possibly of infectious origin. As a basis for the investigation, it was assumed that various infectious agents might be responsible for the condition, and that their toxic products might induce a hypersensibility of certain tissues or structures of the body. If this assumption were true, the specific hypersensibility might be manifested by the skin when properly tested by the causative toxic agent. Therefore the problem was approached by making careful studies of the bacterial flora of all possible infectious foci in the patient. In each instance, autogenous vaccines were prepared from the organisms isolated from these sources, standardized, and by means of intradermal tests it was determined whether or not the patient showed a skin hypersensitivity to them. Those organisms which gave positive skin reactions were selected for therapeutic purposes in the treatment of the patient.

Among the foci of infection, the cervix has been suggested as of much importance in certain cases of chronic arthritis in the female where instances of the disease are relatively high. In a preliminary report submitted to this Society two years ago, this particular focus was considered, and the results of our observations on a series of approximately seventy-five consecutive cases were discussed. Cultures had been taken, under aseptic precautions, from the os and high up in the vagina, and planted in duplicate on blood agar slants, "hormone" agar, and dextrose broth. One set was grown aerobically and the other anaerobically. By that method, approximately 20 per cent of the cases yielded no bacterial growth from the cervical material. As that method of culturing had not proved satisfactory, the media which were used in the earlier studies were discontinued, and during the past two years, the Rosenow dextrose-brain-broth medium has been employed with much better results. In the present series of cases, in only one instance out of thirty-five, no growth developed from cervical material, and in no instance did we fail to get a growth from the vagina in thirty-eight cases. Therefore it has become the routine procedure to use this particular medium and no other in making cultures of materials from the cervix and vagina. It is especially valuable in the culturing of the streptococcus and the enterococcus groups. In this report the bacteriological findings from both the cervix and vagina are combined, as owing to their intimate anatomical relationship. much similarity occurs between their respective flora, although marked distinction between the two not infrequently occurs. The organisms isolated in the order of their frequency from these combined sources were as follows:

Staphylococcus albus (all types)	76 + %
Streptococcus (all types	66 − %
Enterococcus (all types)	55 + %
B. coli (all types)	42 + %
Staphylococcus aureus (all types)	16 − %

Diphtheroid bacilli were occasionally found; the tetragenous in one case; Gram-negative coccus, one case; micrococcus albus type, one case. As noted,

the number of bacterial species isolated from this region was quite limited. From one to six varieties of organisms were cultured from the cervix in the present series of cases.

As stated in the previous report, again the intradermal tests gave variable results with bacterins prepared from apparently the same bacterial species when recovered from distant foci of the same patient. For example, a staphylococcus aureus derived from the cervix gave a three plus (+++) reaction, while one from the sputum gave a negative reaction; a staphylococcus albus from the cervix also gave a three plus (+++) reaction, while one from the nasopharynx gave a negative reaction. On the other hand, a close correspondence between the reactions often occurred from like organisms from distant foci. This interesting biological distinction was previously suggested as offering an especially refined method of studying varieties and subspecies of an organism in particular instances by means of the skin test in the hypersensitive patient.

REPORT OF THE LYMPHATIC TUMOR REGISTRY. George R. Callender, Washington, D. C.

Abstract. At the last report to this Society 100 cases had been contributed to the Lymphatic Tumor Registry of this Association, the period covered being approximately four years. In the past year sixty-three cases have been received, a rate of increase of 250 per cent. I wish to take this opportunity to thank the members of this Association for this increased support. The response to my plea is very gratifying, but the rate of receipt of these cases is still far below what it ought to be. Very few of the cases received during the past year have been cases in which the diagnosis was easy.

The greatest value can be reached from this Registry only if the maximum effort is made by those registering cases to follow the cases to their conclusion, or over a sufficient number of years to ascertain the character of the condition. I believe the difficulties in diagnosis and prognosis of this class of tumors are due to the fact that individuals and groups do not follow up their cases. I further believe that we shall be able to offer definite assistance in diagnosis when and only when we have a sufficient number of cases which have been so followed up as to determine their nature and the changes which occur during the course of the pathological processes.

Please make an additional effort to send in particularly your doubtful cases.

The accompanying table presents a summary of the first 150 cases in the Registry. This summary is not intended as a classification, but it does show the divisions found to apply to those cases so far received.

TUMOR REGISTRY, 1930

Lymphosarcoma	
No mobilization in blood	16
With leukemia (acute)	1
Lymphoblastoma (confined to lymphatic structures)	
No mobilization in blood	0
With mobilization in blood	15
Leukemic	6
Reticulum cell sarcoma	22
(One case with lymphatic leukemia)	

Hodgkin's Disease		
Cellular	10	
Sclerosing	18	
Sarcomatous	12	
Mycosis fungoides (and Tb.)	1	
Lymphoblastic erythrodermia	1	102
Myelogenous leukemia	4	
Myeloma	1	
Chloroma	3	
Infectious mononucleosis (?)	2	
Undiagnosed	14	
Lymphoepithelioma	5	
Carcinoma	12	
Inflammation (1 Tb.)	7	48
		150

Discussion

(Dr. William Boyd, Winnipeg.) I should like to say that in the classification of these tumors there are remarkable differences of opinion. Just a week before leaving Winnipeg I received a report of a case which we sent to the Registry some time ago in which the initial lesion was in the nasopharynx; there were large glands in the neck, and the patient finally died. There were three opinions sent to me from the Registry. The first was that the case was a reticulum cell type of lymphosarcoma; the second, from another man, that it was a transitional cell carcinoma, and the third, from still another man, that it was a case of acute Hodgkin's disease. These three opinions came from men high up in this special field of pathology, but I was left very much in doubt about the case, and I should like to ask Dr. Callender how my case is to be classified.

(Dr. Callender, closing.) As we have no classification, the Registrar, who is really the clerk of the committee, makes a temporary decision which is based on the classification shown there this morning.

HISTOLOGICAL STUDIES OF THE THYROID GLAND. J. William Hinton (by invitation), New York City.

Abstract. Since the question frequently arises as to what constitutes normal and abnormal thyroid tissue, we have obtained for study sections from people whose deaths were due to accidental causes. These cases vary in age from stillbirths to 89 years. As a means of comparison there are also sections from people who died from acute and chronic diseases.

By Jan. 1, 1930, sections from 107 cases had been studied. It was intended to get sections from a large series of cases, rather than serial sections on a few cases, as these would represent more nearly the pathological reports that are received from operative specimens. So far there have been eighty cases of accidental death from which sections of the thyroid have been studied. There are also twenty-seven cases from patients dying from some systemic disease, and it was thought best to divide them into two groups: (1) cases of acute diseases, patients dying in less than three weeks from the onset of the disease; and (2) chronic diseases, those extending over months or years. There were sixteen deaths due to acute diseases, but the sections in this group failed to reveal any

definite histological changes. There were eleven patients who died from chronic diseases and no striking changes were found in the thyroid in this group.

It was stated that sections were taken from people of various ages, and a rather large group has been studied in the hope of finding a uniform histological picture for normal people of different ages. So far no conclusions have been drawn as to what is normal tissue for the different ages, and I must disagree with Hertzler when he states: "For practical purposes I am convinced that if one keeps in mind the various changes normal to the different periods of life as above indicated, the histology of the thyroid gland will be found to be fairly constant, rendering it unnecessary to assume that it undergoes changes unknown in other organs."

In studying patients with thyroid diseases, one is at times unable to place the case in one of the groups given for the classification of goiter, such as adolescent, adenomatous or exophthalmic goiter, and one frequently sees cases change from one type to another while under observation. From this histological study it would seem also that a diagnosis made purely on the histological picture without taking into consideration the clinical history and laboratory data is also open to error, and it seems that one will be forced to consider the different types of goiter merely as a stage of a continuous disease and not a definite clinical entity, as has been believed in the past.

Discussion

(Dr. G. McC. Robson, Philadelphia.) This paper has interested me greatly. Dr. MacFarland and I have reported a study of a series of 100 thyroids secured at autopsy from individuals of various ages, dying of various diseases, acute and chronic (*Arch. Path.*, 1929, **7**, 628-639). We found just what has been reported here — that there was no correlation between the structure of the thyroid and age, sex, color or morbid condition of the patient. The variations which we observed were even greater than those in the slides presented here.

PSEUDO TUBERCULOSIS OF THE THYROID GLAND. Plinn F. Morse, Detroit, Mich.

Abstract. The favorable prognosis which has heretofore been attached to so-called tuberculosis of the thyroid has been the subject of frequent comment by various writers. It has been known that although the thyroid gland operated upon for some type of goiter might show on microscopic examination large numbers of so-called tubercles, the patients usually run a favorable course and react in the usual manner to the thyroid operation, without later succumbing to tuberculous infection, either in the thyroid region or elsewhere.

The cases of so-called tuberculosis of the thyroid have usually failed to present tuberculous lesions in other parts of the body, and the postoperative search for other signs of tuberculous infection has been negative.

The cases that have been diagnosed at the Harper Hospital as tuberculosis of the thyroid have run a favorable course, and all of the patients are at present living and well, several years postoperative, with the exception of one patient who died seven years after operation from acute intestinal obstruction — cause unknown.

The histological study of the lesion seems to justify the assumption that probably the epithelioid reaction resembling tuberculosis is a result either of some other infection, or some metabolic disturbance which causes the colloid

of the gland to assume the rôle of a foreign body. By a study of our cases here, I am impressed that the tubercle results as an effort to absorb and wall-off colloid which has presumably undergone some change which makes it a foreign body. On the basis of these arguments, I am inclined to think that the question of tuberculosis of the thyroid should be left open for further review, in that strong doubt is cast upon the assumption that the Koch bacillus is the etiological factor.

Discussion

(Dr. A. S. Warthin, Ann Arbor.) I should like to ask Dr. Morse if any of these patients have been subjected to radiation. We have had a large number of patients with pseudotubercle of the thyroid of this nature in our material, and in all the cases except two, the thyroids had all been subjected to radiation. As a result of the irradiation, the acinar cells are necrosed, and the colloid acts as a foreign body excitant, and foreign body giant cells appear. While true tuberculosis of the thyroid is more common than one would suspect in the literature, these pseudotubercles are very common. I am quite sure in most cases they are the result of radiation.

(Dr. David Marine, New York City.) I should like to ask Dr. Morse whether he said guinea pig inoculations had been made, or not.

(Dr. Alfred Plaut, New York City.) When I read the title on the program, I expected to hear something about infection of the thyroid gland with the bacillus of pseudotuberculosis. The name "pseudotuberculosis" is meant for an infection with this bacillus. It would be better, for the sake of nomenclature, to call the lesions which Dr. Morse described, tubercle-like foreign body reactions in the thyroid gland.

(Dr. Ginsburg.) I should like to ask what were the clinical symptoms, and what were the diagnoses before operating on these patients.

(Dr. Morse, closing.) As to Dr. Warthin's question relative to the radiation, I do not know about that. That matter could be looked up from the histories, but it had not occurred to me.

As to the question of guinea pig inoculation, my point was that this is the weak place in our investigations. We have not done animal inoculations, and the nature of our material makes it impossible. In some laboratories where frozen sections are done routinely, such work might be productive.

I agree with Dr. Plaut that "pseudotuberculosis" is a bad term, and the condition should be called a tubercle-like foreign body reaction.

The clinical symptoms were those of the various adenomas of the thyroid, — the various forms of toxic thyroid that come into a routine thyroid clinic for operation.

CHRONIC THYROIDITIS. Charles L. Connor and (by invitation) H. H. Searls, San Francisco, Calif.

Abstract. The designation "thyroiditis" dates back to the early days of Kocher. His students, and surgeons in general, retain the name. There is some doubt, however, among pathologists as to whether such a condition actually exists. We wish to present a selected group of cases which, for want of a better name, have been classified as chronic toxic thyroiditis, and to illustrate a possible way in which they might develop.

It has long been known that acute infections and intoxications may cause degeneration of the thyroid epithelium and acini, and that as a result colloid

and cell débris are spilled into the interstitial tissues. This phenomenon is usually regarded as terminal, but it is evident from a study of the literature and some twenty-five cases of thyroiditis in patients entering the University of California Hospital that minor infections or intoxications, perhaps even emotional shock and over-stimulation of the sympathetic system, may induce a similar degeneration of glandular elements. Since such patients do not die, there is an opportunity for a reaction to take place, and this is usually in the form of lymphoid cell infiltration or proliferation. Plasma cells and monocytes may be present, the latter sometimes in the form of giant cells. Pseudogiant-cells are formed by masses of colloid which contain the degenerate nuclei of epithelial cells.

The most constant picture is a rather diffuse desquamation of epithelium, the nuclei appearing in the previously contained colloid within the acini, and a diffuse infiltration with lymphocytes. These latter cells, as well as plasma cells, seem to arise in situ, as evidenced by the number of mitotic figures present in them. Stages in the degeneration of thyroid epithelium appear to be a swelling with fatty degeneration, desquamation and loss of nuclei, and later a breaking down of the substantia propria, so that agglomerations of cytoplasmic masses occur. These eventually disappear, but the process in some cases becomes progressive and prolonged even after (so far as can be determined) the initial stimulus has disappeared. This tendency to progression gives rise to the condition known as chronic thyroiditis, and with great overgrowth of lymphocytes and the concomitant proliferation of fibroblasts, the condition known as Riedl's struma may be the end-result.

Coincident with the degeneration of thyroid cells a more or less active regeneration usually takes place. This may be insufficient to take care of physiological demands, and so symptoms of hypofunction or even myxedema may occur. Or there may be a sufficient regeneration, and we get the histological picture of a moderately hyperplastic gland (associated with thyroiditis), but no symptoms of thyroid dysfunction. This regeneration may proceed to any degree, and a group of our cases exhibit the signs of thyroiditis (pain, pressure on the trachea, hoarseness, etc.) along with the vague symptoms of hyperfunction; and a last group show histologically a marked overregeneration and hyperplasia, associated with frank and definite symptoms of exophthalmic goiter. During this process of regeneration the lymphocytes gradually become less diffuse, become clumped, and in late stages definite lymph follicle formation occurs — the "lymphoid exhaustion" picture of Dr. Warthin. A further stage, conjectured only, would be a frank hyperplasia of the thyroid with characteristic lymphoid cell accumulations and without signs of thyroiditis or cellular degeneration. It would seem that a small proportion of typical thyroid hyperplasias might develop in this manner. It would be unwise, however, to infer that any considerable number pass through these stages.

Discussion

(Dr. A. S. Warthin, Ann Arbor.) I should like to ask Dr. Connor about the administration of iodine in these cases. Most of them impress me as being examples of iodine treatment.

(Dr. E. T. Bell, Minneapolis.) I want to ask Dr. Connor what the relation of this exudative type of thyroiditis is to Riedel's struma, ligneous thyroiditis.

(Dr. Connor.) In answer to Dr. Warthin's question, there was never any

iodine given these patients, except the last few who had definite symptoms of hyperplasia. These few patients were given Lugol's solution; the others were not.

Riedel's struma apparently is a type of chronic thyroiditis, and some of these cases could have been called Riedel's struma.

(Dr. Warthin.) May I ask about the possibility of these patients having taken goiter cures? In numerous cases in which the thyroid presents evidence of the overuse of iodine, the physician has stated that the patient has had no iodine treatment, only to discover later that the patient had been taking a "goiter cure" containing iodine for many months.

(Dr. Connor, closing.) I cannot answer that; I do not know.

STRUMA OF OVARY. Alfred Plaut, New York City.

Abstract. Three specimens of ovarian struma (from three hospitals) were studied. The time elapsed since the operations was too short for definite clinical judgment. The ages of the patients were 47, 35, and 55 years of age. The gross aspect of the specimens added nothing new to the descriptions in the many reports in the literature. In all three specimens, other tissues were found besides the thyroid tissue, thus confirming the generally accepted opinion that ovarian struma is a teratoma thyreoideale. In two of the specimens all the tissues were benign; in the one from a woman 47 years of age, a large portion of the tumor was solid and gave a picture of carcinoma in the gross, as well as microscopically.

The thyroid character of the ovarian struma was obvious in all three specimens. The staining qualities of the contents of the follicles were typical. The three differently colored types of secretion were found with the staining method of E. J. Kraus. Papillary protrusions with high cylindrical epithelium and epithelial buds with high cells were present, giving the picture often seen in struma of a thyroid gland. It is possible to compare the structure of the malignant tumor in the one specimen with the rare type of trabecular adenoma of thyroid gland as described by Masson. The fact that this tumor was gelatinous did not preclude the possibility of its having a common origin with a thyroid-like structure. True mucin has been described in adenoma of thyroid by Nikolsky and by Wegelin. This possibly common origin is further suggested by the fact that mucin-producing solid tumor and typical thyroid struma are intimately mixed in large areas. And, furthermore, there are follicles in which the mucin and the typical thyroid secretion are found together. The ropy appearance of the mucin in the fixed tissue, together with the red color of the mucicarmine form a distinct contrast with the homogeneous, pale blue-staining thyroid secretion. It must be stated that the structure of the carcinoma-like tumor is similar also to some ovarian carcinomas, but the variety of ovarian carcinoma is so great that there is hardly any carcinoma which cannot be compared with one or another type of ovarian cancer.

All three specimens were examined for iodine. The method of Kendall and Richardson (*J. Biol. Chem.* 1920, **43**, 161), was used. In one specimen, four analyses showed no iodine, while controls with normal thyroid gland gave figures from 1.70 to 1.88. In the second specimen 0.025 mg. of iodine were found per gram of dried ovarian struma. This figure compares with the figures of Robert Meyer (0.014 mg.) and of Neu (0.02 mg.). In the specimen which contained the carcinoma-like mass, the thyroid-like tissue and the solid carcinoma-like tissue were examined separately. Two analyses of the thyroid-like tissue showed 0.673 and 0.634 mg., while the solid, cancer-like tumor gave only 0.004. Con-

trols with normal thyroid showed 2.652 and 2.116 mg. Further controls were made with a carcinoma of the breast and an adenocarcinoma of the uterus. Both were negative. The figure 0.673 seems to be the highest for the iodine content of an ovarian struma ever obtained, and it seems high enough to help in asserting that these thyroid-like ovarian tumors can behave functionally in a way similar to thyroid gland. The fact that one specimen, in spite of looking exactly like the others under the microscope, contained no iodine whatever, is certainly important. No difference could be noted in the Mallory stain. Most of the examinations for iodine that have been reported in the literature do not reveal any iodine in the ovarian struma. In the face of the positive findings, however, it did not seem admissible to draw definite conclusions from the frequent absence of iodine. The microscopic pictures were not suggestive of embryonic thyroid tissue, which may be found free from iodine.

Some of the cystic cavities in the specimens contained material which looked different from the clear, brownish yellow thyroid secretion in the struma-like portions. This material gave no reaction for pseudomucin. None of the specimens contained structures which could be compared with a pseudomucinous or serous cyst adenoma. Glandular structures, as may be found in any ovary which is examined in many sections, were present. No statement can be made about their possible connection with the ovarian strumas.

The older literature on the subject deals mainly with the problem whether or not the thyroid-like tissue originated in the ovary itself. This question is settled. There remains the problem: Why does thyroid tissue occasionally grow to such large masses in the ovary? This problem is similar to the one concerning the predominance of the skin, teeth and bone in the dermoid cysts. The findings of Erwin Bauer that in his specimen the struma started from the surface epithelium, are obviously correct, judging from the pictures given in his paper. The literature deals mainly with the general conclusions Bauer has drawn from his serial sections and justly disapproves of them. But the fact remains that ovarian struma can originate from the surface epithelium. With the exception of Walthardt's three specimens, no complete serial sections are reported, and therefore nothing is known about the relations between surface epithelium of ovary and ovarian struma.

The frequent presence of ascites is clinically important, because generally ascites together with an ovarian tumor is taken as a sign of bad prognosis, while ovarian struma, in most instances, has proved to be benign.

BLOOD VESSEL INVASION IN ADENOMAS OF THE THYROID GLAND. Shields Warren, Boston, Mass.

Abstract. From 1923 to 1927 inclusive, thirty-four cases of adenomas of the thyroid showing definite invasion of blood vessels by clusters of tumor cells, but lacking any other evidence of malignancy, were found among the thyroid material received from the Lahey Clinic. None of these cases was suspected of malignancy clinically. Two cases showed recurrence, one in three months and one in ten months, and both patients died with multiple lung metastases, respectively ten months and two years after operation. The other thirty-two patients are living and well, from seven to two and a half years after operation, except for one patient dying of carcinoma of the uterus two and a half years after a thyroid operation. Thus, blood vessel invasion may be the only sign of malignancy in a small proportion of adenomas of the thyroid gland.

SKELETAL METASTASES IN CARCINOMA OF THE THYROID. Isaac Levin, New York City.

Abstract. This study represents a continuation of several years of investigation of the pathogenesis of skeletal metastasis in carcinoma. The formation of these metastatic tumors in the bone, in the opinion of the author, depends not so much on the channels of transportation of the tumor emboli, as on the interaction between the proliferation of the transported tumor cells and the protective resistance of the adjoining normal tissue.

The significance of the thyroid in this study is due to the phenomenon that presumably benign tumors of the thyroid, and even normal thyroid tissue may form metastases.

From the clinical standpoint, the importance of the subject lies in the fact that the whole clinical symptom complex may be due to the skeletal metastatic tumors, while the primary carcinoma in the thyroid may cause no symptoms. In the cases reported, the condition in the bone was considered clinically to be primary, and the diagnosis was made only on the biopsy material.

NOTES ON CERTAIN OF THE SO-CALLED SARCOMAS OF THE THYROID. Lawrence W. Smith, New York City.
(*Abstract not received.*)

THE THYMUS GLAND IN TOXIC AND NON-TOXIC GOITERS. Alfred S. Giordano, South Bend, Ind.
(*Abstract not received.*)

THE INTERACINAR EPITHELIUM OF THE THYROID GLAND. Alan Richards Moritz, Cleveland, O.

Abstract. By means of silver impregnation of the periacinar reticulum and wax plate reconstruction of thyroid gland, it is shown that new acini are formed by intra-acinar and extra-acinar proliferation of epithelium. The extra-acinar proliferation is in the form of small solid or tubular buds which, when cut tangentially, may have the appearance of rests of undifferentiated interacinar epithelium. In hyperplastic areas of normal glands, and in glands the seat of pathological hypertrophy and hyperplasia, a labyrinthine intercommunication of acini exists.

Discussion

(Dr. William Boyd, Winnipeg.) Three different schools of thought are represented in this paper. The first school stresses the importance of the interacinar epithelium. The more one studies these pathological thyroids, the more one is convinced of the budding outwards, as well as the inward budding of the acinar epithelium. I have some slides I would like to show taken from the lesion called fetal adenoma. I think one can see the budding in this type of lesion much better than in the hyperplastic thyroid of Graves' disease. In a section like this one can trace the proliferation of the lining cells with greater ease than in cases of Graves' disease. The two slides show the same picture, but are from different patients.

THE SIGNIFICANCE OF THE LYMPHOID TISSUE IN EXOPHTHALMIC GOITERS AND SO-CALLED TOXIC ADENOMAS. Aldred Scott Warthin, Ann Arbor, Mich.

Abstract. Exophthalmic goiter and "toxic adenoma" always present the pathological picture of hyperplasia of the primitive lymph nodes of the thyroid, hyper-

plasia of the thymus, and the other anatomical stigmas of the thymiclymphatic constitution. In addition, they present certain constitutional peculiarities of their own kind. Not all cases of thymiclymphatic constitution will present the Graves' disease syndrome, although all cases of the latter will possess the chief anatomical stigmas of this constitution. All forms of Basedowian or Graves' symptoms represent the abnormal reactions of a primary pathological anomaly. Basedowian or Graves' disease, "toxic goiter" and "toxic adenoma" are pathological reactions potentially predetermined in the individual at birth, by virtue of his constitutional anomaly. The potential Graves' constitution may be recognized histologically in the thyroids of very young children. To the underlying pathological and clinical entity of exophthalmic goiter, toxic goiter and toxic adenoma, I have applied the term "Graves' constitution."

Discussion

(Dr. David Marine, New York City.) I quite agree with Dr. Warthin in regard to Graves' disease, that there is an anlage, a constitutional defect behind it. However, I cannot agree that they are all born with Graves' disease, and think the constitutional defect, which closely resembles that of status lymphaticus, can be acquired, and also can be congenital or hereditary. The acquired form develops around the menopause. It has something to do with insufficiency of the suprarenal cortex and the gonads. The older view spoke of Graves' disease complicated by status lymphaticus. I think that the lymphoblastic overgrowth is an integral part of Graves' disease. We can readily produce this lymphoblastic overgrowth by removing the suprarenal gland in any of the laboratory animals. Suprarenalectomy causes complete thymic regeneration in the rabbit and rat, and also hypertrophy of the lymphoid tissue in other parts of the body.

(Dr. H. L. Jaffé, New York City.) I should like to ask Dr. Warthin whether he found lymphoid hyperplasia in the thyroid in patients who were not toxic and were iodized.

(Dr. D. P. Seecof, Cleveland.) This is in line with Dr. Jaffé's question. In Colorado I was impressed by the large number of cases that had been iodized thoroughly, but on gross examination of the thyroid coming to the laboratory, I would diagnose it hyperplasia, because the thyroid was dense, grayish white, and showed no colloid. The picture was exactly as Dr. Warthin described it, and if it were not for the few groups of acini scattered through the gland, one would think he was dealing with a lymph node. But I cannot agree with Dr. Warthin regarding the difference in amount of lymphoid hyperplasia in the adenoma (the toxic adenoma) with or without symptoms. I could find no difference between the amount of lymphoid hyperplasia present in the adenomas from patients with, or without, symptoms.

(Dr. L. W. Smith, New York City.) The Lahey Clinic is very much interested in this question. In some 2000 cases of thyroid disease which we had the opportunity to section, we tabulated the cases, and grouped the lymphoid involvement on the bases of +, ++, +++, and ++++. In over 40 per cent of the cases of hyperthyroidism it is perfectly true one finds this lymphoid hyperplasia; in the remaining 60 per cent no hyperplasia is found. As this stimulated our interest, I took a series of normal thyroids, or at least thyroids removed routinely during the course of autopsies for other diseases, and in those instances we were able to find lymphoid hyperplasia throughout the thyroid gland in

15 per cent of the cases, so while I agree with the major premise of Dr. Warthin that there may be a constitutional idiosyncrasy with respect to this lymphoid relationship and hyperthyroidism, I do not feel it is quite as sharp and clear-cut as he would indicate.

(Dr. Warthin, closing.) There are patients with Graves' disease who go all their lives without any symptoms. We would expect to find a certain number of cases showing lymphoid hyperplasia where the patient never develops exophthalmic goiter or toxic adenoma. Those cases which the gentleman from Boston mentioned are cases of quiescent Graves' disease, a potential Graves', but they may never develop it clinically. They have the possibility of having clinical Graves' disease, but they may never have it; as in the case where the patient went fifteen years, but still showed the lymphoid hyperplasia, and I am sure still has a big thymus. That is no argument against my theory, but only helps out my view.

In regard to Dr. Marine's statement of it being acquired, I do not believe the thymicolymphatic constitution is ever acquired. It is always congenital.

THE ESSENTIAL THYROID CHANGES IN GOITER. David Marine, New York City.

Abstract. Perhaps the greatest difficulty in understanding and classifying the essential changes in goiter has arisen from the fact that the thyroid is a very labile tissue, capable of rapid progressive, and even more rapid regressive, changes in all periods of life. In the life history of most goiters there are many of these cycles, and obviously, in a gland normally composed of retention cysts, each succeeding cycle becomes morphologically more complicated and more difficult of interpretation. This tissue lability and the long duration of goiter have led to the inclusion, as Virchow pointed out seventy years ago, of a great variety of secondary and terminal degenerative changes with the essential changes.

The clue for their separation was furnished by Baumann's discovery of iodine as a normal constituent of the thyroid. The extensive studies on the relation of the morphological changes to the iodine store, in both the naturally occurring and experimentally produced goiter which resulted from this discovery, have made it possible to work out a scheme of the sequence and relative importance of the major features of the thyroid cell and to separate the primary from the secondary changes.

Another cause of confusion has been the attempt to bring the morphological features into specific relationship with prevailing clinical classifications of human thyroid diseases, rather than to bring the clinical classifications into relation with the morphology, which is clearly a more constant and more natural standard. Thyroid hyperplasia is analogous to leucocytosis and has no more specific relation to metabolic diseases than leucocytosis has to infectious diseases.

Comparative studies have shown that the essential changes in goiter are similar in all the species of animals studied, and that they can be arranged in a comparatively simple scheme. When large series of human thyroids (autopsy) are studied in the light of the comparative pathology, the same cycle of changes is found so that one can arrange these morphological changes in a scheme that adequately presents the type and sequence of the changes which compromise the cell cycle in goiter.

1. *Normal:* The thyroid is so labile that to insist on too strict a definition of "normal" invites quibbling and defeats the object of establishing a practical standard. If the gland does not exceed 25 gm. in weight, if the follicles are filled

with colloid, if the iodine store is at least 0.2 mg. per gram of fresh tissue, and if the epithelium is low cuboidal, we have an adequate standard of normal.

2. *Hypertrophies and Hyperplasias:* All the work that has been done, whether with experimental regeneration following partial removal or the spontaneous or experimental production of goiter, shows that thyroid enlargement begins as a work hypertrophy and hyperplasia, and that in their developmental stages they are essentially the same for all animals. The sequence of events appears to be as follows: There is a decrease in iodine store, a decrease in the stainable colloid, an increase in the blood supply and a change in the epithelium from low cuboidal to high cuboidal and columnar. All gradations of this series of changes are observed from the earliest departure from normal to the extreme overgrowths of the marked hyperplasias.

Considerable confusion has arisen because pathologists have not always borne in mind the fact that progressive and regressive changes may succeed each other in rapid order and that the progressive and regressive changes are highly variable, both in degree and duration. Thus most goiters undergo hyperplasia and involution many times during their life history and each of these cycles entails certain morphological differences which may be misleading when too great emphasis is laid on any one detail. Thus, variations in the stroma, variations in the size of the follicle and colloid content alter the blood supply and possibly the nervous control which in turn modify secondary regeneration by giving rise to patchy or insular hyperplasia.

3. *Colloid Goiter:* Colloid goiter may be defined as the involution or as the return of an active hyperplasia to the condition nearest to normal, physiologically, chemically and anatomically, that a gland which has once been actively hyperplastic can assume. As is now well known, involution induced by iodine administration is identical with that occurring spontaneously. There is nothing degenerative or atrophic in the process of involution, although such glands may be the seat of highly degenerative or atrophic changes. Complete proof of this is established by the fact that colloid goiters regenerate as readily as normal glands following partial removal or when placed under a potent goitrogenic influence. The processes involved in the formation of a colloid goiter are the reverse of those involved in active regeneration or hyperplasia; that is, the blood supply decreases, the iodine store rises, the epithelium becomes cuboidal and the colloid increases in density. The development of a colloid goiter by the passive dilatation of normal thyroid follicles, I believe, is impossible.

4. *Exhaustion Atrophy or Sclerosis:* While involution to colloid goiter or physiological recovery is the usual mode of termination of compensatory hyperplasia, it occasionally happens, especially in endemic cretinism and in exophthalmic goiter, that the maximum degree of hyperplasia reached and the maximum capacity for function fail to bring about physiological compensation, and sooner or later a state of thyroid exhaustion supervenes, that is, atrophy in spite of attempts at regeneration. The essential changes appear to be analogous to those seen in atrophic cirrhosis of the liver; that is, they are the result of long continued hyperactivity and injury without sufficient physiological rest. In the first stages the appearance of such a thyroid differs very little from any other extreme degree of hyperplasia, save for the increase in stroma. Later, desquamation, atrophy and necrosis result. The cells become highly variable in size and staining intensity. The nuclei also become irregular, sometimes enlarged, hyperchromatic and sometimes pyknotic. The colloid is greatly reduced and as the process continues the follicles are reduced to nests of irregular cells in a dense stroma.

5. *Struma Nodosa:* One of the secondary features of goiter in man requires especial notice, namely, the development of nodules of glandular tissue in long-standing human goiters, so-called adenomas. These growths have something in common with true tumor and also usually retain many of the physiological attributes of normal thyroid tissue. Knowledge of these growths is at present very inadequate. The fact that they are limited almost entirely to human goiters renders experimental approach difficult. That they are an integral part of diffuse goiter is shown by the fact that all nodular goiters, in their earlier stages, were diffuse goiters. This is shown in Wegelin's well known study. As to their origin, the idea of Woeffler that they arise from fetal rests, and the unfortunate term "fetal adenoma" introduced by Billroth have so influenced thyroid pathology during the last fifty years that it is difficult to undo the harm which this conception has introduced. While one cannot deny that occasionally congenital adenomas may arise from fetal rests, there can be no question that practically all of them arise from fully differentiated thyroid tissue and that their origin is intimately bound up with repeated hyperplasia and involution in long-standing goiters. This process, by altering the blood supply and nervous control, and through pressure effects, leads to insular, irregular and localized hyperplasias which eventually become so independent as to lose some of their physiological attributes and take on certain of the characteristics of tumor.

6. Lastly, I would like to call attention to the efforts Wegelin has been making to arrive at an acceptable international classification of the principal stages of goiter. If the principal features could be grouped in some acceptable way, the intermediate or subgroups could be left largely to individual fancy without endangering our ability to understand what is meant. It seems to me the following classification would include the essential major divisions in their sequential relations:

I. Struma diffusa
   1. Struma diffusa parenchymatosa
   2. Struma diffusa parenchymatosa et colloides
   3. Struma diffusa colloides
   4. Struma diffusa sclerosa (exhaustion atrophy)

II. Struma nodosa (adenomatosa)
   1. Struma nodosa parenchymatosa.
   2. Struma nodosa parenchymatosa et colloides
   3. Struma nodosa colloides
   4. Struma nodosa sclerosa

A Study of the Islands of Langerhans in Vivo. Benjamin N. Berg, New York City.

Abstract. The islands of Langerhans were studied in the pancreas of the living white mouse under amytal anesthesia. Microscopically, they appeared as brilliant yellowish white bodies on the surface of the less refractile yellow pancreas, or attached to veins. Accurate observations were possible only when the islands were situated at the surface of the pancreas. Great variations were encountered with respect to the number of islands that were superficial.

In suitable preparations the circulation in the islands was distinguished clearly. The capillaries appeared as thin, thread-like loops that had U, S, V or spiral shapes and dipped into the substances of the glands. The circulation was extremely rapid and changes were noted in the capillary pattern. Rhythmic spontaneous contractions were observed in the small arteries.

Observations were also made concerning the action of epinephrin and pituitrin on the blood vessels of the pancreas, including the circulation in the islands of Langerhans. Vasoconstrictor effects were noted with both drugs. In the higher orders of concentrations, blanching and cessation of the circulation in the islands were observed. With more dilute solutions, the effects upon the circulation in the islands were irregular and difficult to follow. Pituitrin was tolerated by the mice in much higher concentrations than epinephrin.

The results of the studies with epinephrin and pituitrin indicate that the circulation in the islands of Langerhans is probably regulated by changes in the afferent arterioles or the small arteries in response to physiological stimuli.

(*a*) THE SPLEEN IN SUBACUTE BACTERIAL ENDOCARDITIS OF THE VIRIDANS TYPE. (*b*) SYSTEMIC CLASSIFICATION OF SPLENIC PATHOLOGY. Herbert Fox, Philadelphia, Pa.

Abstract. (*a*) Despite the extensive literature on this clinical subject there are no general descriptions of the morbid anatomy of the spleen. This article summarizes the findings in twenty-five acceptably diagnosed cases. The main features of the results are as follows.

The most important gross lesion is the infarct. Practically every case shows perisplenitis, whether an infarct exists or not.

In early stages the organ is not greatly enlarged, but suggests a soft splenic tumor of the congestive type. In the later stages the organ is definitely enlarged, due to hyperplasia of its constituent tissue and to the inflammatory and hyperplastic changes incident to infarctions.

Hyperplasia of lymphatic elements proper is not a feature of this disease; on the contrary those tissues are inactive. Hyperplasia of cells of the reticuloendothelial series is not seen early but appears more evident as the disease progresses.

Marked disease of the blood vessel lining is not an outstanding peculiarity of this disease. Evidences of blood destruction are missing in early, but present in older cases. Degenerations and coagulation within germ centers are frequently observed.

Structures suggesting Bracht-Wächter bodies have been seen several times. Clasmatocytes are occasionally seen but rarely in any number. Polynuclear neutrophiles are very prominent in spleens of this series of bacterial endocarditis cases. Eosinophiles are also frequently seen.

However, if we approach the scoring from the direction of the average change in each of the microscopic tissues, some light can be thrown upon the location in which the major changes are occurring. Instead then of making a sum of the score for each case, the clinicopathological groups are tabulated and their respective values averaged, the relative values for different diseases may become apparent. The group diagnosed as Banti's disease can be used as an example of the computation; they are listed in order of their duration.

60....	1	1	2	3	2	2	2	3	2	2	3	2	1
49....	3	1	2	3	3	2	2	3	2	2	2	2	1
3....	3	3	1	3	2	2	2	3	2	1	2	2	2
37....	1	1	2	3	2	2	2	3	2	3	2	1	1
56....	1	1	2	3	2	2	2	3	1	3	3	2	2
	9	7	9	15	11	10	10	15	9	11	12	9	7
	1.8	1.4	1.8	3	2.2	2	2	3	1.8	2.2	2.4	1.8	1

From these results the major tissue changes in Banti's disease are dilatation of sinuses and fibrous tissue overgrowth, well known features of the disease. But this emphasizes the inactivity of the splenic lymphoid elements and the reticulo-endothelial system. Pigment is shown to be increased. In the group of splenic anemia, secondary anemia and hematemesis, we find follicular and connective tissue, and reticulo-endothelial cells contributing to the splenomegaly.

In the hemolytic anemias, blood, reticular cell increase and fibrosis are the causes of enlargement. The purpura spleen has as its dominant change, sinus dilatation, bloodiness and reticular cell increase; on the whole it is an inactive spleen. In those cases in which the splenomegaly is the dominant feature of the case and there is no hepatomegaly, all parts of the spleen may be actively increased, but this is most marked by the high scoring of follicles, reticular cells, sinusoid structures and fibrous tissue. This is different from the cases in which enlargement of both organs exists and it seems to be independent of which organ enlarged first. The contrast is not great but is sufficient to note. In combined splenic and hepatic enlargement, blood vessel changes, sinus endothelial increase and fibrosis are much more marked than in the simple splenomegalies, which are for the most part inactive in lymphoid tissue, sinus endothelia are very prominent, reticular cells easy to find and fibrosis the highest scoring feature.

In the spleen with lymphocytosis, of which we have but two examples, there are lymph tissue atrophy, dilatation of sinuses, increase of fibrous tissue and richness of blood.

In splenomegaly accompanied by polynucleosis there are prominence of lymphoid tissue and excess of blood, while the pulp and its contents seem inactive.

The tissues contributing to the enlargement of the organ in subacute bacterial endocarditis are the sinus endothelium and the sinus size, blood and adventitious neutrophiles.

(*b*) The difficulty of giving a name or classifiable description to many spleens that are chronically enlarged, received from operation or autopsy, suggested the possibility of grouping them according to a system based on numerical values given arbitrarily to the principal anatomical features. The thought grew from a request made by a surgical colleague to score spleens as epithelioma is scored. The idea was first rejected as impossible but, an attempt being made, the writer found that a rough grouping did occur in two relatively easily separated groups, — one showing splenomegaly, hematemesis and fibrosis, and the other showing splenomegaly, purpura and pulp hyperplasia without lymph element participation. Mathematicians assured the writer that if the anatomical features adopted represented the correct selection and if the changes adopted were correct, it was acceptable to build formulas upon their digital arrangement or on their sum. This is by way of answering the question of the numerical value of a quality, because the changes in splenic pathology would be as much qualitative as quantitative.

A large chart was made, putting into columns all the gross characters of the whole organ and its section surface, all the characters seen under the microscope. If within normal limits they were evaluated at 2; if inactive or atrophic or less than normal their score was 1, and if larger, more active or hyperactive in anyway, the figure was 3. To this were added columns for adventitious changes such as infarct, which were graded 0 or 1. It was found shortly that scoring of whole organs or section surfaces led to no grouping whatever, whereupon scoring was confined to microscopic findings.

The features were reduced from an original charting of 20 to 13, and this will have to be reduced still further. Sums of the numerical values of the characters do not permit distinct groupings of the spleens of the various forms of splenic anemia, nor do these scores differ sufficiently from those of purpura or bacterial endocarditis. In the limited group of viridans cases there is a rough separation of the spleens of acute and those of more protracted cases. Thus, the more acute cases range from 12 to 15, while the more chronic examples range from 14 to 19.

The only contribution this work can offer now is that an attempt is being made to schedule organic changes in the spleen in a numerical formula with the hope that some grouping will appear. The next steps are two —the formulation, around main characters, of the changes in less variable ones, and the comparison of numerical formulas assembled according to the clinicopathological diagnoses. Attempts to show that deductively, spleens will fall into groups that have a pathological significance have not been discarded. The features used are the following: follicles and germ centers, pulp and its splenocytes, sinuses and their endothelium, blood vessels, number and condition of wall, fibrous tissue, recticulo-endothelial cells, blood, pigment, neutrophiles and eosinophiles. Adventitious characters do not receive numerical values.

A [Comparison of Four Lines of Mouse Leukemia, Transmitted by Inoculation. Maurice N. Richter and (by invitation) E. C. MacDowell, New York City and Cold Spring Harbor, N. Y.

Abstract. In previous communications we have reported the presence of an inbred strain of mice in which lymphatic leukemia occurs with great frequency, and the fact that the leukemias occurring spontaneously in this strain may be transmitted to normal young mice of the same strain by inoculation with emulsions of tissues.

From each of the spontaneous cases used as donors for the transmissions, a line of inoculable leukemia was obtained, transmissible apparently indefinitely by inoculation.

In these lines of experimental transmissions we have observed varieties of manifestation similar to those occurring in spontaneous cases. Some manifestations, however, have occurred so frequently in certain lines, and infrequently in others, as to indicate the presence of differences in the inocula prepared from each.

The transmissions of the several lines were carried on in parallel experiments, the host animals all being from the same inbred strain, and frequently from the same litter. Consistently reappearing differences in the lines are not, therefore, due to differences in the hosts.

Discussion

(Dr. J. Furth, Philadelphia.) We have also succeeded in transmitting leukemia of mice. By intravenous inoculations leukemia is produced in a higher percentage of experimental animals than by intraperitoneal inoculations, and it may be transmitted even to a strain of mice in which spontaneous leukemia has not been observed. This necessitates some modification of the genetical theory of McDowell and Richter on susceptibility to transmitted leukemia. In about twenty-five days after the inoculation, about 20 per cent of the inoculated mice developed a systemic enlargement of the lymph nodes, followed a few days later by the involvement of the circulating blood, leukemia produced by intravenous

inoculation being very similar to spontaneous leukemia and to Line I of Richter and McDowell. It would seem that if leukemic cells are introduced into the circulation, they are filtered out, and only after a certain degree of lymphoid hyperplasia has been attained do they invade the blood. Leukemia and aleukemic lymphadenosis are apparently essentially the same process, a conclusion that Richter and McDowell have likewise reached.

(Dr. Powsler, New York City.) It would seem that this leukemia is inheritable from one generation to the next. The offspring have a liability to leukemia.

(Dr. Richter). We first began systematic observations in the eighteenth generation of this line. It is now in the twenty-third generation.

(Dr. Powsler.) It is an inheritance due to some characteristic of some other organism, let us say white blood cells, or to some other condition which causes the organism to act that way; the point is that it is not a true genetic characteristic, but is inherited in the same way as syphilis, for example.

(Dr. Richter, closing.) In reply to Dr. Furth, the inoculation of other lines has been attempted, and in our experience has been negative so far. We have not attached very much importance to this because it has been recorded already that leukemia occurring in mice may be transmitted to other strains of mice. However, I feel that in the experiments of Furth the low percentage of "takes" may be explained by the fact that the mice into which the inoculations were made were not inbred, and therefore not uniform.

With regard to Dr. Powsler's question, we feel that the reappearance of leukemia in this strain is on a genetic basis, and we base this not only upon the appearance of leukemia in this strain, but on the ratios of susceptibility in hybrids which we have observed. Some of these observations were reported at the American Association for Cancer Research. It is not due to transmission through the mother, because hybrids with a leukemic male parent, and a non-leukemic female parent have also been susceptible. It is apparently not analogous to the so-called "inheritance" of syphilis or similar conditions.

EXPERIMENTAL OSTITIS FIBROSA (FIBROUS OSTEODYSTROPHY) IN GUINEA PIGS ON NORMAL DIET, INJECTED WITH PARATHORMONE. H. L. Jaffé and (by invitation) A. Bodansky and J. E. Blair, New York City.

Abstract. It has been suspected for about twenty-five years that the parathyroids are in some way related to the bone dystrophies. Recently Mandl, and after him others, removed enlarged parathyroids in cases of ostitis fibrosa cystica and reported rapid improvement of their patients, with cessation of the negative mineral balance.

No reports have appeared on the experimental production of the fibrous osteodystrophies by the use of parathyroid extract. Nor have bone changes that fulfill the requirements for the diagnosis of ostitis fibrosa been produced experimentally by other means.

By subcutaneous injection of parathormone we have regularly produced in the guinea pig the generalized bone changes which satisfy all the criteria of ostitis fibrosa, including the appearance of new bone (osteoid). The latter appears as soon as the reparative processes are permitted to operate with sufficient intensity. A guinea pig permitted to go for a few days without parathormone, after previous parathormone treatment, shows an abundance of osteoid tissue beneath the periosteum and endosteum, in the haversian canals, and in the fibrous tissue in the metaphysis just distal to the epiphyseal cartilage plate.

It was necessary to use relatively large doses of parathormone to produce fibrous changes in the bones of guinea pigs. The doses can be given to the guinea pig without producing fatal hypercalcemia, and the animal's ability to withstand such large doses may be related to the alkaline diet of this animal.

Even one dose of 60 units given to a guinea pig weighing 300 gm. produces extensive resorption, cessation of bone formation and infraction of the cortex within forty-eight hours. Such guinea pigs may die with extensive bone destruction, if the large doses are continued. We produced bone changes in guinea pigs with as little as 10 units of parathormone daily, and with 20 to 30 units daily for two or three weeks we obtained very extensive resorption and fibrosis of the bones.

In the dog, if the doses are large enough to produce rapid bone changes, fatal hypercalcemia may result. However, we have been able to prevent hypercalcemia in dogs on as much as 20 units of parathormone daily and have produced in these dogs generalized bone resorption with lacunar erosion, fibrosis, cysts and osteoid tissue — all criteria for the diagnosis of ostitis fibrosa cystica.

Discussion

(Dr. Paul Klemperer, New York City.) I think this excellent paper is a very important contribution to decide the long debated question whether the parathyroid hyperplasia so often found in osteitis fibrosa generalisata is the cause or the result of the calcium deprivation of the skeleton. Previous experience, namely the observation of parathyroid hyperplasia in generalized bone carcinomatosis, has always caused me to consider the hyperplasia the result, and not the cause of the bone destruction, but I feel the presentation of Dr. Jaffé and his co-workers speaks entirely in the other direction. It is of course very important to decide this question, since surgeons here and abroad are starting to resect the hyperplastic parathyroid in order to cure the osteitis fibrosa. From the pictures presented, it seems as though Dr. Jaffé had been able to reproduce all the stages of osteitis fibrosa, both the hyperostotic as well as the hypoostotic forms. I should like to know whether Dr. Jaffé has any ideas about the relationship of these experiments to the localized forms of osteitis fibrosa, the brown tumors in the bones, and the bone cysts in isolated parts of the skeleton.

(Dr. Jaffé, closing.) We have not particularly made up our minds about the localized osteitis fibrosa. However, a great number of conditions are really included under localized osteitis fibrosa, many of which are purely inflammatory lesions, which leave as a residue fibrous changes in the bone, because for some reason or other, the bone has not reconstructed itself normally. It appears to us quite possible that there may be one bone osteitis fibrosa, and at the present time we are inclined to view the lesion as due possibly to a lowered threshold, local in that bone, possibly on the basis of circulatory disturbances, which makes that bone peculiarly and particularly susceptible to the fibrosis-producing factors.

THE HISTIOGENESIS AND DEVELOPMENT OF RETICULUM; ITS WIDESPREAD OCCURRENCE IN THE ADULT ORGANISM. A New Method of Demonstration. James F. Rinehart (by invitation), San Francisco, Calif.

Abstract. A method of impregnating connective tissue substance, including reticulum and collagen, is given in which there is apparently a complete impregnation of such substances and in which adequate counterstaining is achieved.

The mesenchyme is shown to possess a fibrillar cytoplasm very closely related to reticulum and collagen. The differentiation of capillaries in loco in the mesenchyme is shown; the differentiation of mesenchyme into adult connective tissue is also indicated. Reticulum is shown to be a direct descendant of the mesenchyme, and evidence is presented of the probable transformation of reticulum into collagen. Reticulum fibrils are demonstrated as of universal occurrence in the capillary endothelium. Reticulum is identified with the basement membrane in the kidney, gastric mucosa and pancreas. The epithelioid cells of tuberculosis are shown to be capable of forming reticulum fibrils which may be transformed into collagen. By securing complete impregnation of this fiber substance and combining it with adequate counterstaining, a more complete concept of the capillary bed is achieved in which reticulum-lined spaces are shown to connect apparently isolated capillaries, as seen by ordinary staining methods.

RAPID DIAGNOSIS OF INTRACRANIAL TUMORS BY SUPRAVITAL STUDY. Louise Eisenhardt (by invitation) and Harvey Cushing, Boston, Mass.

Abstract. Description of a method of immediate diagnosis of intracranial tumors by supravital study of fresh smears is given. The method has been in use for the past two and one-half years and it is possible by this means to differentiate the various types of gliomas, for example, almost more easily than by the usual methods of fixed preparations.

Discussion

(Dr. W. C. MacCarty, Rochester, Minn.) I have been devoting the last twenty-five years of my life to the study of living tissues, and especially unfixed tissues, and should be among the first to congratulate Dr. Eisenhardt on this work. I was very much interested, for I visited Dr. Cushing's laboratory about a month ago and saw these slides in actual preparation. I can say also, in spite of the fact that these slides are very beautiful, that they do not compare with the originals from which the slides were made. I do not know just how many pathologists appreciate just what this service is to a surgeon, but those of us who have been intimately connected with surgical work know that he will act on the nature of the tumor. He must know the type of tumor; he cannot wait even for an hour, because an hour with a patient under anesthesia means something in brain surgery, so I hope you will take seriously this service which Dr. Eisenhardt has rendered, and apply the method. The method which she has shown is somewhat different from that which I have used in the last twenty-five years, but the end results are the same, and that is the important thing after all. This method gives much more beautiful results than anv fixed method I know of.

(Dr. William Boyd, Winnipeg.) Can this method be used for autopsy material? Can it be used only on fresh, living material?

(Dr. Eisenhardt, closing.) These preparations are made only from fresh living material as soon as possible after it has been removed from the body. We have not attempted to use the method on autopsy material.

AN EXPERIMENTAL STUDY OF THE EFFECTS OF HEAT INDUCED BY HIGH FREQUENCY ALTERNATING CURRENTS. V. C. Jacobsen and (by invitation) K. Hosoi, Albany, N. Y.

Abstract. About two years ago it was noted in a laboratory of radio research that when a workman happened to get in the field of a short wave radio trans-

mitter, a rise in his temperature occurred, the height of temperature depending on the time he was in the field and his proximity to the apparatus. A rise of 2.2 degrees was caused in fifteen minutes and the response was very pronounced when a water-cooled 20 kilowatt radio tube was discharging from a six foot rod to ground with 60,000,000 alternations per second, of 15 kilovolts. Since diathermy with its relatively low rate of alternation of current is reputed to have valuable therapeutic applications due to the heat generated in the tissues, Dr. W. R. Whitney, director of the Research Laboratory of the General Electric Company, devised a modification of the radio transmitter which has become available for the study of experimental aseptic hyperthermia. The apparatus is a high frequency heater or oscillator on the principle of a short wave transmitter, except that the energy is concentrated between two plate electrodes instead of being directed from an aerial. Complete details of the machine are being published elsewhere. In the experiments I shall report, a wave length of 25 meters was used almost entirely, an amperage of 0.2 to 0.35, oscillating about 10,000,000 per second, a rate so rapid that no muscular contractions are produced.

Twenty-three dogs, twenty-one rats and four guinea pigs were exposed between the plate electrodes for variable periods of time. A temperature once reached could be maintained either by decreasing the voltage or by increasing the distance between the electrodes. A dog's temperature taken by rectum, or determined by thermocouple, could be raised as much as 7.1 degrees in thirty-seven minutes, 8.3 degrees in less than an hour, such rapid heating often causing death, but not always. Three dogs were kept at about 109° F from five to twelve hours. Twelve dogs were given from two to nine heatings that lasted from four hours to thirty hours, the temperatures varying from 107° to 112° F.

The white rat and the guinea pig could withstand about the same temperature as the dog and the tissue changes are practically the same for these three species under the same conditions of the experiment.

After removal from the oscillator, the temperature with few exceptions would return to normal in three hours. Repeated exposures would rarely give indication of loss of control of the heat-regulating center as shown by a prolonging of the pyretic state.

While being heated, the animal would become restless, salivate, pant, perspire, and the visible membranes become congested.

In some dogs placed too close to the electrodes, perspiration caused arcing, resulting in severe skin burns which healed very slowly. Rapid heating of rats often caused a disruption of the skin of the tail and severe burns at the base of the tail, with the skin finally sloughing off.

Postmortem examinations were performed as promptly as possible since postmortem degeneration was very rapid in the highly heated tissues.

The animals that died or were killed while still at a high temperature showed the following conditions:

Acute congestion of all the organs. In addition, the heart showed focal hemorrhage, interstitial edema, fatty degeneration of muscle fibers. The lungs showed emphysema, atelectasis, small hemorrhages and increased secretion of bronchial mucus. Hemorrhages and necrosis of lymphoid tissue were present in the spleen; endothelial hyperplasia was noted. Often fibrin thrombi were present in the sinusoids. The gastro-intestinal tract showed a few mucosal hemorrhages with necrosis of lymphoid tissue, excess mucus secretion, mesenteric hemorrhage, and an acute inflammation of mucosa was often a coincident

infection. Cloudy swelling, fatty degeneration, focal necrosis, focal hemorrhage, and depletion of glycogen were present in the liver. The pancreas was normal. The kidneys showed cloudy swelling, hydrops, fatty degeneration in Henle's and convoluted tubules. Focal hemorrhage, increased visible fat, hemorrhage in the capsular fat were seen in the adrenals. Dogs' testes were usually normal. Rats' testes showed much exfoliation of germinal epithelium and proliferation of Sertoli cells.

One female bulldog heated to 108° F for nineteen hours had a breast carcinoma which appeared unaffected.

The striated muscle showed glycogen depletion. The bone marrow was hyperactive. The thyroid was normal. Congestion, focal hemorrhage and chromatolysis of ganglion cells was noted in the brain. Chromatolysis of ganglion cells was present in the spinal cord.

Dogs and rats heated many times and the temperatures allowed to return to normal showed fewer changes in their organs. Congestion was regularly present but cloudy swelling and fatty degeneration were less frequently seen. Loss of weight was pronounced and the fat depots showed loss of fat. The weight was usually recovered rapidly, however, by the drinking of water. The pulmonary emphysema in the animals given one heating was probably due in part to an acidosis of lactic acid nature and not to ketosis.

The heat induced by this method is an internal heat, probably caused as Hosmer suggests, by an increased vibration of molecules of the cells produced by their alternate attraction to each of the electrode plates in turn.

From a study of the tissues of these experimental animals, it is seen that there is little to suggest any fundamental difference between the heat induced by this oscillating current and the hyperthermia in febrile disease, or that produced by external applications. Concomitant pulmonary or gastro-intestinal infection was present in slight degree in only a few animals. The dangers of the method are not entirely known. The cutaneous burns were preventable. Schereschewsky found experimental mouse cancer unfavorably influenced by this form of heating. Its application to various chronic diseases such as arthritis and paresis seems logical and such work is under way.

The pathology of fever can be studied more thoroughly than ever before by this method and yet its clinical application must be preceded by much fundamental work, since acidosis, and later alkalosis and tetany, cloudy swelling and fatty degeneration of parenchymatous organs, and deleterious effects upon the germinal epithelium of testes and ovaries are definite pathological states to be avoided in therapy if possible.

Finally, it seems quite in order in this anniversary year of Dr. William H. Welch to say that his Cartwright lectures given in New York in 1888 still constitute our greatest source of information concerning the pathology and etiology of fever, little of note having since been added.

Discussion

(Dr. Robert Koch, Pittsburgh.) At the Western Pennsylvania Hospital in Pittsburgh, Drs. Szymanowski and Hicks are experimenting on the biological effect of short wave radiation. The experiments show very clearly that radiation of this kind produces other effects besides the heat effect. In complicated systems like the animal body, the heat effect is so pronounced that other effects are masked. Simple systems like collodial solutions or diphtheria toxin offer a better means for studying all the biological effects of short wave rays.

SPONTANEOUS AND EXPERIMENTAL SCHWANNOMAS. Pierre Masson and (by invitation) Charles Simard, Montreal, Can.

Abstract. Nageotte has shown that if a fragment of the sciatic nerve of the rabbit is excised from one side and grafted near the intact sciatic nerve of the other side, a voluminous tumor is produced by the sheaths of Schwann without participation of the axones — an artificial schwannoma. Comparative study of one of these tumors and of several spontaneous encapsulated neurinomas from man, shows a complete identity of their fundamental constituents. The structure, mode of collagen production, the manner of growth and development are the same in both types of tumor. A study of their characters shows that these constituents are different from connective tissue cells. In the building up of experimental schwannomas, connective tissue plays an important part, but in encapsulated spontaneous schwannomas the connective tissue plays only a minor part. The pure connective tissue aspect (fibromatous, myxomatous) of certain neurinomas corresponds to special morphological modification of Schwann cells which have been altered by subjection to certain circulatory conditions. The palisades that are found in many spontaneous schwannomas are derived from the sheath of Schwann and are perhaps characteristic of the type of tumor derived from sensory nerves.

QUANTITATIVE OBSERVATIONS ON THE SEMILUNAR VALVES OF THE HEART. Paul Gross (by invitation), Cleveland, O.

Abstract. A method for the determination of the surface area of valves is described. The surface area of the cusps of a semilunar valve with a large ring is commensurately greater than that of a valve with a normal ring. Valves with normal and large rings present no significant differences in the height of the cusps. Elongation of the valve rings of hearts at autopsy is associated with a decrease in the height of the cusps. It is probable, therefore, that as a valve ring increases in size the cusps increase in height. The surface area of the semilunar valves is 40 to 70 per cent in excess of a calculated minimal closing surface. The percentage of excess has no correlation with the size of the valve ring. A semilunar valve is incompetent if the average height of the cusps is equal to or less than the radius of the valve ring.

STUDIES ON THE PATHOGENESIS OF BACTERIAL ENDOCARDITIS. Robert Koch and Kurt Semsroth (by invitation), Pittsburgh, Pa.

Abstract. Injections of casein or killed streptococci, a prerequisite for the development of bacterial endocarditis in our experiments, lead neither to degenerative changes of the endocardium nor to a transformation of the endothelial coat of the endocardium into phagocytes. Experimental studies on the vital staining of the cells of the reticulo-endothelial system, however, reveal that subsequent to injections of casein or killed streptococci the reticulo-endothelial system loses to a considerable extent the ability to develop granular deposits of dyes, if the dyes are injected in the form of *solutions*, while the phagocytosis of *colloidal* particles of the same dyes is not disturbed.

Experiments were carried out to determine whether or not the functional disturbance of the reticulo-endothelial system, reported above, influences the degree of the deleterious effect of toxic bacterial substances on the heart. Control rabbits were given doses of a diphtheria toxin or typhoid vaccine which did not lead to inflammatory reactions of the endocardium nor to a myocarditis. Analo-

gous doses were given to rabbits that had received previously a series of injections of casein or streptococci vaccine. These animals developed a focal interstitial myocarditis. Furthermore, proliferative and exudative inflammatory reactions of the endocardium with marked degenerative changes were observed. It is inferred that the functional disturbance of the reticulo-endothelium system reported in the preceding paper is associated with an increased vulnerability of the heart to the action of bacterial products.

CHARACTERISTICS OF STREPTOCOCCI ISOLATED FROM PATIENTS WITH RHEUMATIC FEVER AND CHRONIC INFECTIOUS ARTHRITIS. R. L. Cecil and (by invitation) E. E. Nicholls and W. J. Stainsby, New York City.

Abstract. During the past three years, streptococci have been isolated frequently from the blood and joints of patients with acute rheumatic fever and chronic infectious arthritis. The technique consists in taking large quantities of blood and observing the cultures over a long period of time.

83.3 per cent of the strains isolated from cases of chronic infectious arthritis appear to belong to one biological group. These strains have been designated by us as "typical strains." They seem to fall into the alpha prime group of streptococci.

The streptococci recovered from cases of acute rheumatic fever belong to the *Streptococcus viridans* group although the methemoglobin formation is frequently difficult to demonstrate. Cross-agglutination tests show that some of these organisms tend to fall into biological groups.

Agglutination reactions with the serums of patients with chronic infectious arthritis against the antigen of any of the typical strains are very striking. The serums agglutinate to a very high titer, as high as 1:5120 or more. This condition is not found with control serums. Serums of patients with acute rheumatic fever agglutinate some of the rheumatic fever strains to a higher titer than do serums from control cases.

Discussion

(Dr. Emanuel Libman, New York City.) Dr. Cecil requested me to discuss this presentation. He asked me to state whether or not I was of the opinion that these streptococci came from the air or were in the culture media. I have no doubt that they came from the blood of the patients.

I will not go deeply into the controversial side of the subject under discussion. My main purpose is to draw attention to the tremendous amount of time and energy that often has been lost in connection with the bacteriological study of streptococci in general — and the non-hemolytic streptococci in particular.

In the first place, most of the investigations of the fermentation reactions of these organisms are of little value as the more favorable media have not been employed. Many studies have been performed with agar media without the addition of serum. In the course of elaborate investigations made many years ago, we found that this medium is the poorest for the purpose. The next best is a bouillon medium without serum. Agar plus serum is much better and bouillon plus serum is still more favorable. Even the last-mentioned is not an optimum medium. The Hiss serum-water medium is of little value unless peptone is added (as first suggested by Buerger). Even then it is not nearly as good as the bouillon-serum method. For over twenty years I have been convinced that we must be careful of classifying non-hemolytic streptococci by means of their fermentative power on various carbohydrates.

My second point refers to agglutination, complement fixation, and other serological reactions. Many investigators have employed bacteria grown on media containing serum. Dr. Cecil, in the studies reported to-day, has avoided this error.

It was reported by Olitsky and Bernstein in 1916, and by Olitsky and Denzer in 1917 that rabbits injected with bacteria grown on media containing serum responded by producing antibody against the organisms and against the serum proteins. Such immune sera are capable of giving non-specific reactions with various other bacteria cultivated upon the same kind of serum. Even after the fourth washing (saline solution) yields no evidence of serum proteins, the same result may ensue. These studies have been confirmed by F. S. Jones. In a paper published last year by Hooker and Anderson in the *Journal of Immunology* (a preliminary report of which appeared in 1924) this subject is well emphasized. In the course of a very elaborate and careful investigation of 616 alpha streptococci, cross-agglutination reactions were found in less than 3 per cent. The non-hemolytic streptococci are very individualistic in their characteristics, and undergo changes rather easily. I would not lay stress, for example, on the slight differences found by Dr. Cecil and his co-workers in the streptococci obtained in rheumatic fever, and those found in the cases of infectious arthritis.

What is the present status of the question of the etiological relationship of streptococci to rheumatic fever?

There are facts that speak for and against it. The final proof is not yet forthcoming. We know that similar streptococci are found in cases of subacute bacterial endocarditis and in the occasional instances of mixed infections (both active) of the two conditions. We know that they may be found in the blood without endocarditis appearing, in severe or mild local infections. What is most important is the fact that they may be found as transitory invaders in the greatest variety of febrile and non-febrile disease. In the last two cases of Hodgkin's disease that I have observed, non-hemolytic streptococci were present in the blood. In the second case, three positive cultures were obtained by Dr. Goldzieher (to whom I am indebted for permission to cite this case to you). In lymph nodes from a number of diseases, non-hemolytic streptococci have also been found in a viable condition. The studies of E. Z. Epstein and M. A. Kugel, that non-hemolytic streptococci may be cultivated in nearly all cadavers from the spleen and bone marrow, and in the heart muscle as well as valves that show no endocarditis, in as high a figure as (approximately) 50 per cent are of great significance.

It would have been of advantage if febrile cases had been employed as controls in the studies just reported. The fact, however, that the investigations revealed so few positive results in the fever-free control cases, except for those of chronic infectious arthritis in which they were so numerous, compels our attention.

(Dr. B. J. Clawson, Minneapolis). It seems to me this is pretty nearly the last link in the chain in confirming the belief that streptococci are the causal agent in acute rheumatic fever. The fact that these organisms are found in the joints is important; if they were found only in the blood, they might be secondary invaders, but when we have them present in the joints in such a high percentage of cases, it is pretty hard to think we have a secondary invader. It is also true of the organisms in the blood; those of you who have attempted to culture the blood of patients who are not as ill as those with acute rheumatic fever, find it

quite unusual to demonstrate streptococci in the blood. It is true with Hodgkin's disease, and late in carcinoma it is a common thing to find streptococci in the blood. But with people who are not very ill it is quite unusual to find streptococci in the blood. I have followed Dr. Cecil's work very carefully, and I should like to ask if he has come to any conclusion as to why the organism grows so slowly at first, and then after you get it out on proper culture media, it grows so easily.

(Dr. V. H. Moon, Philadelphia.) I am greatly interested in this work of Cecil and his associates, and while the subject of the etiology of acute rheumatic fever is under discussion, I should like to remind those who were not here yesterday of some results we have obtained by the inoculation of animals with *Streptococcus viridans* obtained from blood culture and from the heart valves of a case diagnosed clinically as bacterial endocarditis. The inoculation resulted in the formation of the verrucous type of endocarditis on the mitral valve and in the auricle, the formation of nodules in the walls of the blood vessels, in the heart muscle, and in skeletal muscles, and of fibrous nodules histologically identical with the subcutaneous nodules in acute rheumatic fever. I think this should be borne in mind as confirmatory of the evidence of the relationship of streptococci to acute rheumatic fever.

(Dr. Nichols, closing.) In answer to Dr. Clawson's question, we have not found any explanation for the slow growth of the organisms.

HISTOLOGICAL STUDIES ON THE ASCHOFF BODY. Louis Gross and (by invitation) Joseph C. Ehrlich, New York City.

Abstract. The descriptions of Aschoff bodies found in the literature are in many cases confusing. There are many disagreements on the histological appearance of these bodies, mainly because various authors depict different structures as well as different phases in the evolution of the same structure. This also accounts to a certain extent for differences of opinion on the origin of the cells found in Aschoff bodies. By using various histological methods on a large amount of autopsy material, a series of pictures have been found which suggest a fairly definite life cycle for the Aschoff body. In certain cases, at least, the Aschoff body takes the form of a well developed syncytium surrounding swollen collagen as one characteristic phase in the life cycle. Several phases of this cycle appear to be pathognomic of rheumatism. It is believed that if this cycle is thoroughly understood there will be less difficulty in recognizing these structures. The Aschoff body is shown to possess a characteristic reticulum which also appears to go through a recognizable life cycle.

Discussion

(Dr. B. J. Clawson, Minneapolis.) I want to repeat the same question I asked last year, and ask Dr. Gross to give a definition of a typical Aschoff nodule. Another question I should like to ask is how he knows that one end or another is the beginning of the life cycle of an Aschoff nodule. He certainly demonstrated that the Aschoff nodule may take many forms. There may be various stages. Another point about those elongated ones, I should like to know what would happen if they were cut the other way, that is, transversely rather than longitudinally.

(Dr. V. H. Moon, Philadelphia.) I should like to ask whether the eight or nine characteristics which Dr. Gross states must be present for the diagnosis of an

Aschoff body, will be found at an earlier stage of the lesion equally as well, and if they will persist through the later stages of the same lesion.

(Dr. Emanuel Libman, New York City.) I would like to clarify one point. I do not believe that Dr. Gross meant to say that Fahr described only one case. Did he not describe a small endemic? The findings were exceptional, and therefore there is a strong possibility that he happened to be dealing with a group of cases in which scarlet fever and rheumatic fever coexisted.

(Dr. H. F. Swift, New York City.) I would like to point out in connection with what Dr. Libman has said that Siegmund only recently has reported a large series of cases where death occurred several weeks following the acute stages of scarlet fever, and stated that as the time lengthened between the onset of scarlet fever and the time of death, the final lesions of the cardiovascular system approximated more and more those seen in rheumatic fever; hence the time element appears to be an important factor. As far as I can recall, all of Fahr's cases were fatal soon after the onset of scarlet fever; and when considered in connection with the observations of Siegmund, bring up a most important question in the general consideration of this problem — the time between the onset of the scarlet fever and the occurrence of the peculiar type of lesion. Only after the noxious agent has been active for several weeks or months does the tissue appear to respond in the peculiar proliferative manner observed in either true rheumatic fever or scarlatinal rheumatism and carditis.

(Dr. Herman Schwarz, New York City.) In a case of scarlet fever with joint manifestations, I had the opportunity of examining the heart seven weeks after the onset of the disease. There was a definite double murmur at the apex, but what was more important, I found typical rheumatic nodules on both olecranon processes. It might be concluded that in this case the so-called scarlatinal rheumatism was genuine rheumatism.

(Dr. Gross, closing.) In answer to Dr. Clawson's request for a definition of the typical Aschoff body, may I say I had hoped our demonstration would serve this purpose. However, if Dr. Clawson will tell me of what stage in the cycle he would like to have a definition of the typical Aschoff body, I shall be glad to give it to him. I have tried to point out the fact that the Aschoff body is not a static structure. It is fluid and continually changing. At one characteristic stage, after the syncytium has partly broken down, the cell protoplasm is generally supple, voluminous and basophilic; the cell edges are ragged; there are small masses of naked protoplasm about; there are also masses of swollen collagen and a peculiar "chicken wire" silver-staining reticulum to be seen; the nuclei are vesicular, relatively clear, with a dense perinuclear membrane and a conspicuous dark central nucleolus (owl-eyed). Giant cells may or may not be present. There is no elastica and rarely fibrin to be found. We have not found this picture thus far in any other condition. As I said before, however, my definition will have to vary with the stage of the cycle described.

Like Dr. Clawson, we were also puzzled as to how certain we could be about which was the tail and which the beginning of the cycle. When one tries to work out the life cycle of a lesion occurring in the human, where one cannot have experimental control, there is always an element of uncertainty. But there are certain factors which one can use as a guide, for example, the clinical history, the appearance of fresh foci of polymorphonuclear leucocytes, the appearance of fresh verrucae, the maturity of the verrucae and, best of all, the state of swelling of the collagen and the maturity of the connective tissue. In working out the

life cycle of the Aschoff body we had to be guided not only by such factors as these, but also by how well these factors dovetailed into each other.

With regard to the appearance of a polarized Aschoff body when looked at on end, we have sectioned the Aschoff bodies serially, and at the polarized stage the end view shows a central nucleus surrounded by very scanty protoplasm. The hyaline connective tissue is replaced largely by delicate fibrillae.

Dr. Moon has asked whether these various characteristics persist. Let me answer this question categorically to a certain extent. The protoplasm basophilia appears just before the height of the cycle and goes through to the end. The reticulum starts at the beginning and persists to the end stage, that is, the stage of mature connective tissue when the silver-staining reticulum completely disappears. The nuclei are characteristic at the height of the cycle and continue almost to the very last stages.

With regard to Dr. Swift's remark about the element of time, I agree with him thoroughly of course. We would be illogical if we denied the possible rôle which time may play in this connection, let alone the question as to whether rheumatism is a modification of scarlet fever. It seems to me, however, that at the present time one has no right to assume otherwise than that this disease, for some unknown reason, seems at times to follow on scarlet fever. One would have to have other evidence to link the two diseases under one etiology.

EXPERIMENTAL STREPTOCOCCIC INFLAMMATION IN IMMUNE AND HYPERSENSITIVE ANIMALS WITH SPECIAL REFERENCE TO THE PATHOGENESIS OF RHEUMATIC LESIONS. Benjamin J. Clawson, Minneapolis, Minn.

Abstract. The experiments were performed with the following three groups of animals: (1) animals which had not been injected (normal animals); (2) animals which had been injected intravenously with streptococci (immune animals); (3) animals which had been injected subcutaneously in one area with agar at 45° C, heavily seeded with streptococci (allergic or hypersensitive animals). Each animal was injected in ten places in the subcutaneous tissues on the right side with a 1:100 suspension of streptococci and in ten places on the left side with a 1:1000 dilution of the suspension.

The polyblastic type of microscopic cellular reaction was found in all three groups. No difference could be detected in the character of the reaction in the nodules. It appears evident that the polyblastic type of reaction, which is characteristic of the lesions found in human rheumatic cases, does not depend primarily upon a hypersensitive stage when produced experimentally in animals. If doses sufficiently large are given, this reaction may be produced in both normal and immune animals, as well as in the hypersensitive animals.

The relationship between allergy and the polyblastic type of reaction appears to be a quantitative one. This quantitative relationship may help to explain the pathogenesis of human rheumatic lesions in many cases. Since streptococci have not been found in large numbers in the blood and joints of patients having acute rheumatic fever, it has been difficult to understand the extensive lesions; but when it is taken into account that small doses of streptococci will produce extensive reactions in hypersensitive animals and that high percentages of patients having acute rheumatic fever are hypersensitive to streptococci, the pathogenesis of the human lesions can be understood more readily.

Discussion

(Dr. H. T. Karsner, Cleveland.) The tissue response described by Dr. Clawson in these experiments suggests the possibility that he may be dealing with anaphylactic inflammation as described by Opie. Will Dr. Clawson state whether or not the bacteria were washed for use in sensitization, immunization or subsequent injection?

(Dr. Homer Swift, New York City.) Dr. Clawson's report is more or less in confirmation of what we suggested last year. There is one point well worth emphasizing, namely, the qualitative relationship, the type of response and the type of soil in which the bacterium is planted. Dr. Karsner has brought up a point which also appears well worth keeping in mind, namely, the relationship of bacterial hypersensitiveness to that phenomenon which Opie called anaphylactic inflammation, to which Rössle gave the name of hyperergic inflammation, where the argument is based upon experiments done with a different type of antigen. If one uses for preliminary sensitization a coagulable protein such as egg-white, he obtains after reinoculation into the tissues a hyperergic or an anaphylactic type of inflammation that is increased in intensity more or less parallel with the increase in the concentration of circulating immune bodies. With whole bacterial antigens, that does not hold, as is brought out by Dr. Clawson's work. The hyperergic inflammation following injection of bacteria into the tissues is determined by the preceding mode of inoculation. If it has been into the subcutaneous tissue, and now I am dealing with streptococci, the animal's reactivity is increased, so that a so-called hyperergic type of inflammation results from reinoculation. If the dose of antigen be accurately titrated, and the lesions resulting from various doses be excised daily and compared, not only a histiocytic response will be observed in the hyperergic inflammation, but various types of exudative reaction will be found early. On the other hand, an animal with high antibody content in its serum resulting from previous intravenous inoculation will respond to intracutaneous injection of small or medium-sized doses only with a histiocytic mesenchymal reaction; in the first the response may well be termed hyperergic inflammation, and in the second immune hypo-ergic reaction.

(Dr. Louis Gros, New York City.) In a personal conversation with Dr. Clawson he very kindly informed me of the fact that he had obtained similar subcutaneous nodules in the skin of rabbits injected with hay bacilli. I should like to know whether he was able to discern any histological differences between the nodules produced by the hay bacilli and those produced by the streptococci.

(Dr. Clawson, closing.) I have avoided the term anaphylactic on purpose, for the reason that Dr. Swift brought out. There is a disagreement among immunologists as to just what the hypersensitive reaction, such as the tuberculin reaction, is, and realizing that, I used the term hypersensitiveness. If the hypersensitiveness produced by bacterial proteins is anaphylaxis, then I think this is an anaphylactic reaction. I am not certain it is. It is not the same thing which we get by injecting horse serum. This reaction seems to depend on something in those abscesses. What this is, I do not know, and I am waiting for some immunologist to tell us.

In reply to Dr. Karsner's question about washing the organisms, the first were not washed. I used a broth culture of organisms mixed with agar. The standardized organisms were washed.

The quantitative reaction should be emphasized, and while these injections

were standardized fairly closely, we are now carrying on a more accurate method of standardization. In all these cases, control animals were run with the same doses of organisms each time.

In reply to the question about the hay bacillus, this reaction is not specific for streptococci. We have used the hay bacillus in the same way, and have been able to produce the same reaction. In fact, we have been able to reproduce with the hay bacillus the secondary reaction reported by Swift and his co-workers.

SUBCUTANEOUS NODULES IN CHRONIC INFECTIOUS ARTHRITIS. M. H. Dawson (by invitation) and A. M. Pappenheimer, New York City.

Abstract. The occurrence of subcutaneous nodules in patients suffering from rheumatic fever has long been recognized and has been the subject of intensive investigation by many workers. Less attention, however, has been paid to the subcutaneous nodules that occur in patients afflicted with rheumatoid (chronic infectious) arthritis.

During the past year in the Arthritic Clinic of the Presbyterian Hospital, subcutaneous nodules have been observed in twenty-three patients. All of the patients presented the typical clinical picture of rheumatoid or chronic infectious arthritis. Nodules were excised from eleven of these patients and subjected to careful histological and bacteriological examination.

All the material examined has shown a striking, uniform and characteristic picture. The essential histological features may be summarized as follows:

(1) an area of central necrosis, apparently due in its earliest stages to a gelatinous swelling and degeneration of individual collagen bundles; (2) a surrounding zone of peculiar and characteristically arranged large mononuclear cells; (3) an enclosing zone of dense and relatively avascular fibrous tissue.

The blood vessels in the area of the nodule itself rarely show significant changes, but the arterioles and capillaries in the surrounding tissue are the site of characteristic lesions.

There is a striking resemblance between the histological appearance of these nodules and those which occur in rheumatic fever.

The bacteriological investigations have been entirely negative.

Discussion

(Dr. Emanuel Libman, New York City.) I want to speak because I feel that this valuable contribution, so excellently presented, should not be passed without any discussion. Were any nodules found near the sacro-iliac synchondroses? I ask this question because I have been much interested in the nodules found in that situation by Goldscheider who believes that they are of gouty origin. A thorough study of them has not been made. I feel that they indicate a metabolic disturbance, and that it is still to be proved that they indicate real gout. Goldscheider has found urate crystals in some of them, and believes that when such crystals are not found, they had been present and had been absorbed. It would be interesting to determine whether or not all nodules over the sacro-iliac synchondroses are of metabolic origin. Possibly some may be found to be of infectious origin, or even of combined metabolic and infectious origin.

(Dr. Kurt Semsroth, Pittsburgh.) I was particularly interested in the fact that Dr. Dawson referred to the type of necrosis as gelatinous. If I was not mistaken, the particular type of necrosis, starting with the gelatinous swelling of the connective tissue, has been stressed by Dr. Dawson. When I examined his sec-

tions of the nodules of chronic rheumatoid arthritis, I was struck by the extreme analogy of these degenerative changes with those which we observed in cases of viridans-endocarditis associated with Aschoff bodies. Furthermore, that particular type of gelatinous necrosis of the connective tissue is frequently seen in the vascular lesions in chronic streptococcus sepsis, all of which might lead one to stress the probability of the causative relation of streptococci to these lesions. On the other hand, I do not believe that such a conclusion can be made, since Klinge of Leipzig recently has noticed that very type of gelatinous necrosis in the connective tissue of rabbits hypersensitive to horse serum, and which were subsequently injected into the knee joints with horse serum. He claims to have induced lesions in the synovia, in the periarticular tissue, in the connective tissue of the arteries removed from the site of injection, in the myocardium and endocardium, characterized by the same type of gelatinous necrosis and by proliferation of large mononuclear elements. In the light of these results it appears that a certain state of the host is one determining factor in the pathogenesis of rheumatic lesions. Another, but not the only, factor may in man well be the invasion of the host by streptococci.

(Dr. Dawson, closing.) We have had no difficulty in differentiating any of these cases from cases of gout. We have studied some nodules from gouty cases. and their structure is apparently entirely different.

A New Meningococcus-like Organism (Neisseria Flavescens, n. sp.) from Epidemic Meningitis. Sara E. Branham (by invitation), Washington, D. C.

Abstract. Of 155 strains of meningococci isolated from cases of epidemic cerebrospinal meningitis in the United States during 1928 and 1929, fourteen strains were uniformly atypical. These were among fifty spinal fluid strains received from one city during a single outbreak, and thirty-six of which, or 72 per cent, fell into the four antigenic groups of Gordon's classification. The remaining fourteen, or 28 per cent, formed a homogeneous serological group. There was no cross-agglutination with any of the four usual groups of meningococci; neither was any of these strains agglutinated by polyvalent commercial antimeningococcus sera.

Aside from this serological difference these fourteen uniform strains varied from the typical meningococci in pigment production and in total lack of fermentative ability. They did not correspond with any of the gram-negative cocci previously described.

The cases with which these cases were associated were subacute and ran a relatively long course. Eleven of the patients were given polyvalent antimeningococcus serum freely, and seven made a complete recovery.

This homogeneous group of fourteen strains does not conform to the usual conception of the meningococcus, although the strains were isolated from the spinal fluid of meningitis cases during an epidemic in which all four of the usual serological types of meningococci were found. To call them meningococci would alter the definition of a meningococcus and plunge the classifications of the meningococcus group into confusion. For the present we shall refer to this new group of fourteen strains as *Neisseria flavescens, n. sp.*

The fact that 28 per cent of the strains isolated in one locality, or more than 9 per cent of those received from the country at large, are members of this restricted group suggests the importance of giving special attention to it.

ATTEMPTED CHEMOTHERAPY IN EXPERIMENTAL RABIES. A. Hoyt (by invitation) and C. W. Jungeblut, New York City.

Abstract. A typical and constant infection was produced in white mice by the intracerebral injection of fixed virus. The animals usually showed beginning paralysis in about six days, and died on the eighth or ninth day.

In experiments involving 255 mice, which were injected intracerebrally with 0.02 cc. of different dilutions of virus, the approximate minimum fatal dose was 1:320 dilution of this virus. Though the majority of animals injected with amounts of virus up to as little as 1:1280 died of rabies, a few survived. Beyond this point the percentage of survivals increased rapidly.

Attempts were made to protect mice against rabic infection produced by intracerebral injection of one or more minimum lethal doses of fixed virus, by prophylactic treatment with silversalvarsan, neosalvarsan, tryparsamide, Bayer 205 (Germanin), plasmochin, optochin and quinine bisulphate. The drugs, with the exception of quinine bisulphate, were injected intravenously in doses as large as could be tolerated. Treatment was ordinarily started on the day of the infection; in some cases it was continued by one or more subsequent doses of the drug.

None of the drugs used displayed the slightest prophylactic or therapeutic effect on the infection, under the conditions of the experiment. There was, however, often a slight prolongation of the incubation period of the disease in some of the treated mice, as compared with that of the controls. This was especially noticeable with silversalvarsan and Bayer 205 (Germanin), while the incubation period appeared to be actually shortened by the administration of the quinine compounds. Caution should be used in interpreting the above results, as the incubation period of a few mice, treated with distilled water only, was likewise slightly lengthened.

BLOOD CHANGES DURING TRYPANOSOME SEPTICEMIA. Richard W. Linton (by invitation), New York City.

Abstract. A study of some aspects of the blood chemistry of rats with acute septicemia due to infection with *Trypanosoma equiperdum*, yielded the following results: The carbon dioxide capacity of the serum was markedly lowered early in the disease. The non-protein nitrogen and uric acid constituents in the blood were increased in the terminal stages. The kidneys also showed terminal degenerative changes. The cholesterol remained unchanged throughout. Lecithin was markedly increased, most of the observations showing a 20 to 50 per cent rise in this substance. Liver glycogen was lower than normal in the early stages and could not be demonstrated in the later stages of the infection. The blood sugar remained normal until a very late period in the disease.

READ BY TITLE

COR BIATRIATUM TRILOCULARE, COMPLICATED BY OTHER GRAVE CARDIAC ANOMALIES AND COMPENSATORY DEVELOPMENT OF ANASTOMOSES BETWEEN BRONCHIAL AND PULMONARY CIRCULATIONS WITH FORMATION OF CONGENITAL ARTERIOVENOUS ANEURYSM IN LUNGS. FROM A MAN AGED 20 YEARS WITH COMPLETE CONGENITAL HEART-BLOCK AND DEATH FROM PULMONARY HEMORRHAGE. Maude E. Abbott, Montreal, Can.

MUCOID NEOPLASMS OF THE URINARY TRACT. Nicholas M. Alter (by invitation) and Joseph M. McCarthy (by invitation), New York City.

SARCOMA IN WILD MALLARD DUCKS. David L. Belding, Boston.

THE NEUTROTROPISM IN THE HUMAN TESTICLE AND HYFOPHYSIS. Louis Berger (by invitation), Quebec, Can.

A NEW MENINGOCOCCUS-LIKE ORGANISM (NEISSERIA FLAVESCENS, N. SP.) FROM EPIDEMIC MENINGITIS. Sara E. Branham (by invitation), Washington, D. C. (*cf. Abstracts*).

REPORT OF CASE OF ACUTE LEUKEMIA WITH AUTOPSY. Baxter L. Crawford, Philadelphia, Pa.

EXPERIMENTAL PNEUMOCOCCUS INFECTION IN THE HORSE. Theodore Curphey (by invitation), New York City.

THE BEHAVIOR OF THE COLON BACILLUS AND ITS SPECIFIC BACTERIOPHAGE IN URINE CULTURES. Frances C. Frisbee (by invitation) and W. J. MacNeal, New York City.

ERYTHROLEUCOSIS AND THE ANEMIAS OF THE FOWL. J. Furth (by invitation), Philadelphia, Pa.

METASTASIZING CARCINOID TUMOR OF JEJUNUM. Istvan Gaspar (by invitation), Rochester, N. Y.

STRUMA OVARII. J. L. Goforth, Dallas, Texas.

A FATAL CASE OF GENERALIZED MONILIASIS WITH SPECIAL REFERENCE TO THE PATHOLOGY. S. R. Haythorn and (by invitation) G. H. Robinson and L. W. Johnson, Pittsburgh, Pa.

ATTEMPTED CHEMOTHERAPY IN EXPERIMENTAL RABIES. A. Hoyt (by invitation) and C. W. Jungeblut, New York City (*cf. Abstracts*).

SMALL CELL CANCERS OF THE LUNG. Howard T. Karsner and Otto Saphir, Cleveland, O.

NEOPLASMA OF THE PLEURA — MESOTHELIOMA AND FIBROSARCOMA. Paul Klemperer and (by invitation) C. B. Rabin, New York City.

STANDARDIZATION OF ANTIGEN. B. S. Levine (by invitation), Hines, Ill.

BLOOD CHANGES DURING TRYPANOSOME SEPTICEMIA. Richard W. Linton (by invitation), New York City.

RELATION OF THE FOLLICLES AND THE FOLLICULAR ARTERIES OF THE SPLEEN. W. J. MacNeal and (by invitation) J. M. Ravid, New York City.

STUDIES IN BARTONELLA MURIS ANEMIA. J. Marmorston–Gottesman (by invitation) and David Perla, New York City.

THE EVOLUTION OF MASSIVE PULMONARY TUBERCULOSIS IN THE RABBIT. E. M. Medlar and (by invitation) K. T. Sasano, Mt. McGregor, N. Y.

THE RELATION OF THE EPITHELIUM TO THE MUCOSA IN PACHYDERMIA LARYNGIS. Louise H. Meeker, New York City.

FIXATION OF IRON BY AN INFLAMMATORY REACTION. Valy Menkin (by invitation), Philadelphia, Pa.

THE TOTAL NUMBER OF GLOMERULI IN THE NORMAL HUMAN KIDNEY. Robert A. Moore, Cleveland, O.

FIBROSIS OF LUNG CONSEQUENT TO PULMONARY ARTERIOCAPILLARY FIBROSIS. Eli Moschcowitz, New York City.

CASE OF MYELOMA WITH UNUSUAL AMYLOID DEPOSITION. Beryl H. Paige, New York City.

THE BIOLOGY OF INFLAMMATION. Ernest Pribram, Chicago, Ill.

ENDOCARDIAL POCKETS OF THE LEFT AURICLE AND VENTRICLE. Otto Saphir, Chicago, Ill.

FULMINANT GENERALIZED INFECTION WITH THE PFEIFFER BACILLUS. Adele E. Sheplar and Lawrence Sophian (by invitation), and W. J. MacNeal, New York City.

GLOMERIC TUMOR OF THE SKIN. Charles Simard (by invitation), Montreal, Can.